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JOURNAL OF AGRICULTURAL RESEARCH

VOLUME V

OCTOBER 4, 1915—MARCH 27, 1916

DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

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ERRATA

Page 20, last line, "ammonium sulphate" should read "ammonium carbonate."

Page 22, legend under figure 16 should read "Diagram showing the number of corn plants up and dry matter produced in 21 days on College loam with sodium sulphate, sodium carbonate, and sodium chlorid," etc.

Page 23, legend under figure 18 should read "Diagram showing the number of wheat plants up and dry matter produced in 16 days on Greenville loam with ammonium carbonate, sodium carbonate, and potassium carbonate," etc.

Page 59, "*Dipsacus follonum*" should read "*Dipsacus fullonum.*"

Page 63, "*Capsula bursa pastoris*" should read "*Capsella bursa pastoris.*"

Pages 65, 66, 67, "*Helianthus diversicatus*" should read "*Helianthus divaricatus.*"

Page 174, line 17 from bottom, "32°" should read "30°."

Page 175, Table II, last column, last line, "60.0" should read "67.0."

Page 189, line 13 from bottom, "form" should read "from."

Page 191, line 17 from bottom, "Fusarium Wollenw." should read "Fusarium hyperoxysporum Wollenw."

Page 210, Plate XVII, "figure 1" should read "figure 4," "figures 2, 3, 4" should read "figures 1, 2, 3."

Page 271, line 22, "weight of the kernels" should read "weight of the extracted kernels."

Page 272, line 8, "It was normal" should read "It was not normal."

Page 279, footnote b, "Rabbit 651" should read "Rabbit 951."

Page 291, "*Panax quinquefolia*" should read "*Panax quinquefolium.*"

Page 334, line 11 from bottom should read "thermoelements in this section may be observed."

Page 694, line 7, "2 N 2/5" should read "N 2/5."

Page 700, line 14, "N/5" should read "N 2/5."

Page 752, footnote, line 2 from bottom, should read "For 100 c. c. synthetic solution take 1 c. c. of M/5 magnesium sulphate, 1 c. c. asparagin M/5, and 5 c. c. of each of the other solutions, and add to 88 c. c. water. Steam on three successive days."

Page 780, Plate LVI, figure 2, *B*, "0.1 per cent" should read "0.01 per cent."

Page 782, "*Pinus murrayana* Oreg. Com." should read "*Pinus contorta* Loud."

Page 911, line 13 from bottom should read "and a humid soil after the removal of lime and magnesia."

Page 912, line 10 from bottom should read "10 gm. of dry soil after the removal of lime and magnesia."

Plate LXVI, "Fig. 2" should read "Fig. 1."

Page 986, last line, "also" should read "next to."

Page 987, first footnote, "eighth" should read "seventh."

Page 1016, line 16, "comparing them" should read "comparable."

Page 1023, Table VII, first column, "12.15 a. m." should read "12.15 p. m."

Page 1036, line 4, "spore" should read "pore."

Page 1063, line 3, "4.0065 feet" should read "4.0056 feet."

Page 1071, figure 8 and tenth line from bottom of page, "$C=3.078L^{1.022}$" should read "$C=3.078L^{0.022}$."

Page 1073, line 17, "4.0058 feet" should read "4.0086 feet."

Page 1081, Table VIII, "4.0058-foot notch" should read "4.0086-foot notch."

Page 1083, bottom of page, "$H\left(2.5-\frac{0.0195}{S^{0.75}}\right)$" should read "$H\left(2.5-\frac{0.0195}{S^{0.75}}\right)$."

Page 1095, Table XIV, under "Head, 1 foot," ninth column, tenth line, "4.52" should read "4.53."

Page 1112, Literature cited, "Forschheimer" should read "Forchheimer."

Page 1117, legend under figure 1, end of line 6, "spring" should read "fall."

Page 1187, "22.9" should read "12.9."

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., OCTOBER 4, 1915 No. 1

EFFECT OF ALKALI SALTS IN SOILS ON THE GERMINATION AND GROWTH OF CROPS

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INTRODUCTION

In arid regions the soil is likely to contain an accumulation of soluble salts in such quantities that the growth of vegetation is hindered. Indeed, in many sections the type of vegetation is determined almost entirely by the alkali content of the soil. Every grade may be found, from the soil containing so much soluble salt that no vegetation whatever will grow to the soil containing scarcely sufficient soluble material for the needs of plants.

In the western part of the United States there are millions of acres of land of each alkali type. The worst of these lands need not be considered at present for agricultural purposes, but there are vast areas just on the border line. If everything is favorable, they produce profitable crops; but during the average year crops are a failure. If a permanent agriculture is to be established on these soils, it will be necessary to increase greatly our knowledge of methods of handling them.

A large part of the unsettled land of the West contains more or less alkali. Chemical analysis of the soil can easily be made and the alkali content determined; where the alkali content is very high, the land is not suited to agriculture; where it is low, the alkali can not be considered an interfering factor. It is the soil containing a medium amount that causes the difficulty. Many projects that were condemned when an analysis of the soil was made have proved later to be fertile agricultural tracts. On the other hand, lands whose salt content was thought to be sufficiently low for crop production have later been abandoned. There are not sufficient exact experimental data available to make it

[1] The author wishes to acknowledge his indebtedness to his assistants, Messrs. Howard J. Maughan, George Stewart, and A. F. Bracken, for their faithful and intelligent efforts in conducting certain parts of the work; to Mr. R. M. Madsen, Miss Alma Esplin, and Mr. N. I. Butt for their care in making many laborious computations; and to a number of other faithful assistants who helped in conducting the experiments.

possible in all cases to determine how well crops will grow in a soil of known alkali content.

In view of the great practical importance of the subject as well as its scientific interest, considerably more information should be gathered on the relation of alkali in soils to crops. The limits of endurance of each crop for each salt in the different kinds of soil should be fixed with much greater exactness.

It was in response to this need that the work reported in this article was undertaken.

REVIEW OF THE LITERATURE

The effect on plants of the salts classed as alkali has been the subject of much investigation, but the greater part of this work has been done in solution cultures rather than in the soil. By using water cultures an attempt has been made to limit the great number of factors that exist in the soil, where some of the salts are neutralized and others are absorbed. The work of Loew (16),[1] Kearney (12–14), Harter (7, 14), Cameron (5, 13), Breazeale (1–2, 5), Dorsey (6), Osterhout (20–21), True (26), McCool (18), and others in this country and numerous workers in Europe has added many facts to our knowledge of the action of single salts and balanced solutions on plants grown in water cultures. These workers have shown the great toxicity of salts like magnesium when used alone in a water culture and how this toxicity may be reduced by the presence of other elements.

The facts obtained in these experiments have increased our knowledge of plant physiology and the fundamental nature of alkali; but conclusions drawn from them should not be too definitely applied to the action of alkali as it is found in the soil.

For example, in solution cultures the salts of magnesium when present alone are very toxic, while if added to a normal soil they are no more toxic than a number of other salts. Again, Kearney and Cameron (13) concluded from their work with solutions that "the toxic effect of injurious salts is due very much more to the influence of the cathions (derived from the basic radicle) than to the anions (furnished by the acid radicle)." This may be true for solution cultures, but it certainly does not always hold for salts added to soils, as the results in the present paper will show.

It is desirable, therefore, in studying the effect of soil alkali on plants to use soil as a medium in which to grow the plants, even though it is somewhat difficult to watch all the factors involved.

In 1876 Toutphoeus (9), and Henri Vilmorin (9) about the same time, published results of experiments showing that chemical fertilizers when added to the soil in too large quantities inhibit the germination of seeds.

[1] Reference is made by number to "Literature cited," p. 52–53.

Nessler in 1877 (9) stated that 0.5 per cent of cooking salt (sodium chlorid) injured the germination of rape, clover, and hemp, and that wheat withstands this solution, but is injured by a 1 per cent solution.

Hilgard was a pioneer in the study of alkali soils and as early as 1877 began publishing results on his investigations in California. From that time to the present his contributions, together with those of Loughridge, his associate, have constantly enriched the literature. Their results are contained in numerous publications of the California Agricultural Experiment Station and were well summarized by Hilgard in 1906 (11).

An excellent review of the work done on alkali in the United States up to 1905 is also given by Dorsey (6). A large proportion of the work on alkali in this country has consisted of the analysis of soils for the determination of the presence of various alkali salts.

A number of workers, however, have investigated the amounts of the different salts necessary to inhibit crop growth. Hilgard (10) and Loughridge (17) made numerous studies of the alkali content of California soils and the limits of concentration of the various salts at which cultivated and native plants cease to grow.

Buffum (3), Slosson (23), and Knight and Slosson (15) in Wyoming carried on many experiments on the effect of alkali on the germination of seeds and growth of crops. From their results they concluded that there is a regular decrease in the germination of seeds as the osmotic pressure increases; and there is no apparent difference between sodium or potassium, or between the sulphate and chlorid of the same or different salts. It will be noted that this conclusion is not borne out by the data contained in the present paper.

Headden (8), working with sugar beets, found that varieties differed in their resistance to alkali. He also determined the effect of sodium carbonate, sodium sulphate, and magnesium sulphate on the germination of sugar-beet seed. He concluded that—

> The best seed germinated freely in soil containing as much as 0.10 per cent of sodium carbonate but the plants were attacked by as much as 0.05 per cent and it is doubtful whether any of them can survive when there is as much as 0.10 per cent of this salt present in the soil. Sodium sulphate affects the germination to a much less degree, even when it is equal to 0.90 per cent of the air-dried soil, but it is injurious when present in larger quantities. When both sodium carbonate and sodium sulphate are present in equal quantities, the action of the carbonate, or black alkali, is only slightly or not at all mitigated. Magnesium sulphate retards, but does not prevent germination when present in quantities equal to 1 per cent of the air-dried soil.

Stewart (25) made germination tests of a number of crops in soil to which different quantities of alkali salts had been added. He found sodium carbonate to be the most injurious of the alkalies with most crops. However, with white clover and red clover white alkali proved as injurious as the black. In their resistance to alkali the cereals stood in the following order: Barley, rye, wheat, and oats, barley being the most

resistant. He found that 0.50 per cent of either carbonate or chlorid was fatal to germination in almost all cases.

Hicks (9) found that—

> Muriate of potash and sodium nitrate used as fertilizers in strengths of 1 per cent or more are very detrimental to the germination of seeds, whether applied directly or mixed with the soil; that the chief injury to germination from chemical fertilizers is inflicted upon the young sprouts after they leave the seed coat and before they emerge from the soil, while the seeds themselves are injured only slightly or not at all.

Shaw (22) after a great many tests was led to the conclusion that wherever the chlorid content of soil approached 0.2 per cent beet culture was unsuccessful.

Kearney (12) listed crops most likely to succeed in alkali of various concentrations, as follows: Excessive alkali (above 1.5 per cent), native and foreign saltbush and salt grasses; very strong alkali (1.0 to 1.5 per cent), date palm and pomegranate bushes; strong alkali (0.8 to 1 per cent), sugar beets, western wheat-grass, awnless brome-grass, and tall meadow oat-grass; medium strong alkali (0.6 to 0.8 per cent), meadow fescue, Italian rye-grass, slender wheat-grass, foxtail millet, rape, kale, sorgo, and barley for hay; medium alkali (0.4 to 0.6 per cent), redtop, timothy, orchard grass, cotton, asparagus, wheat for hay, oats for hay, rye, and barley; weak alkali (0.0 to 0.4 per cent), wheat for grain, emmer for grain, oats for grain, kafir, milo, proso millet, alfalfa, field peas, vetches, horse beans, and sweet clover.

Miyake (19), working on the effect of the chlorids, nitrates, sulphates, and carbonates of sodium, calcium, magnesium, and potassium on rice, found that the antagonistic action of individual salts was in part overcome when the salts were combined.

PRELIMINARY STUDIES

RESULTS IN 1912

The study of soil alkali in its relation to the growth of plants was begun by the Utah Experiment Station in 1912. The first tests were made in glass tumblers which held about 200 gm. of soil. The soil used was loam from the Greenville (Utah) Experimental Farm. The chemical and physical analyses of this soil are given in Tables VIII and IX.

The crops were New Zealand wheat (*Triticum aestivum*) and sugar beets (*Beta vulgaris*), 10 seeds being planted in each glass. Each sugar-beet seed, or ball, contains more than one germ; hence, more plants were usually obtained than the number of seeds planted.

The salts were added from stock solutions and were thoroughly mixed with the soil two or three days before the seeds were planted, July 28. The sugar beets were harvested on August 5, and the wheat on August 10. The plants that had come up were counted and their height and dry weight determined. The results are given in Tables I, II, and III.

TABLE I.—*Percentage of germination of wheat and sugar beets in soil containing sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate in different concentrations. Salts added in solution*

Concentration of salts (p. p. m. of dry soil).	Percentage of germination.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None	90	90	90	90	123	123	123	123
100	60	100	80	80	90	50	100	80
500	90	70	40	90	150	40	70	100
1,000	60	70	80	80	170	90	110	120
2,000	40	70	60	90	130	120	120	160
3,000	0	50	50	60	20	100	200	110
4,000	0	50	70	70	0	130	210	180
5,000	0	80	60	50	0	150	250	70
6,000	0	70	60	90	0	90	190	120
7,000	0	30	80	60	0	20	210	210
8,000	0	40	40	80	0	0	150	240
9,000	0	30	70	60	0	0	100	180
10,000	0	0	40	70	0	0	110	210

TABLE II.—*Average height (in centimeters) of wheat and sugar-beet plants raised in soil containing alkali salts in various concentrations*

Concentration of salts (p. p. m. of dry soil).	Average height of plants.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None	24	24	24	24	7	7	7	7
100	24	24	27	25	7	7	7	7
500	24	24	26	21	7	7	7	7
1,000	17	23	26	27	7	7	7	7
2,000	8	23	27	25	3	7	7	7
3,000	.....	22	23	27	3	7	7	5
4,000	.....	22	19	25	.....	6	6	5
5,000	.....	22	21	25	.....	6	6	6
6,000	.....	20	19	26	.....	4	6	6
7,000	.....	10	12	22	.....	3	5	7
8,000	.....	4	10	22	.....	.....	6	5
9,000	.....	3	5	23	.....	.....	4	5
10,000	.....	.....	7	26	.....	.....	4	5

TABLE III.—*Quantity of dry matter (in grams) produced by wheat and sugar-beet plants raised in soil containing alkali salts in various concentrations*

Concentration of salts (p. p. m. of dry soil).	Dry matter.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None.....	0.131	0.131	0.131	0.131	0.020	0.020	0.020	0.020
100.....	.095	.142	.100	.118	.016	.101	.019	.013
500.....	.185	.082	.070	.117	.032	.007	.012	.017
1,000.....	.050	.110	.103	.147	.034	.015	.020	.018
2,000.....	.034	.117	.121	.114	.017	.023	.005	.027
3,000.....	.....	.098	.075	.114	.005	.023	.033	.029
4,000.....	.....	.080	.078	.108	.....	.026	.039	.031
5,000.....	.....	.119	.080	.073	.....	.026	.048	.013
6,000.....	.....	.090	.088	.144	.....	.013	.035	.023
7,000.....	.....	.039	.052	.060	.....	.002	.035	.037
8,000.....	.....	.034	.036	.129	.....	.....	.025	.038
9,000.....	.....	.010	.048	.094	.....	.....	.015	.033
10,000.....	.....	.....	.029	.126	.....	.....	.015	.035

While the data in the tables are somewhat irregular on account of the comparatively small number of plants used, a few facts come out rather clearly. Probably the most conspicuous of these is the relatively high toxicity of sodium chlorid (NaCl) in Greenville soil when compared with other salts.

Two thousand p. p. m., or 0.2 per cent, marked the limit of growth for wheat, while three thousand p. p. m. was the limit for sugar beets. There was germination and growth with considerably more sodium carbonate (Na_2CO_3) than sodium chlorid, although the carbonate dissolved the organic matter from the soil, producing a very bad physical condition. Magnesium sulphate ($MgSO_4$) was only slightly toxic at a concentration of 1 per cent of the soil, while sodium sulphate (Na_2SO_4) was more toxic, but produced fair crops where 1 per cent was present. The percentage of germination, the height of plants, and the dry weight all correspond in showing where the growth began to be retarded by salt.

In order to determine the effect of the percentage of soil moisture on the toxicity of alkali, tests were made with soils having 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 per cent of water on the dry basis. At the one extreme the soil was about as dry as plants would grow in, while at the other it was completely saturated. The soil used was Greenville loam, and the seed planted was New Zealand wheat. The methods were the same as those already described, with 10 seeds in each glass.

The seeds were planted on August 16 and the plants harvested on September 27. The results are shown in Table IV.

TABLE IV.—*Effect of soil moisture on the toxicity of sodium carbonate on wheat plants*

NUMBER OF SEEDS GERMINATED IN EACH GLASS

Concentration of sodium carbonate (p. p. m. of dry soil).	Percentage of soil moisture.							
	12.5	15	17.5	20	22.5	25	27.5	30
4,000.....	8	9	9	9	9	8	7	9
5,000.....	8	8	8	8	7	6	6	10
6,000.....	2	9	9	7	5	9	7	8
7,000.....	4	3	8	5	6	5	3	5
8,000.....	1	8	7	6	4	3	3	4
9,000.....	.....	5	5	5	3	3	1	1
10,000.....	.....	.....	3	5	5	4	3	1
11,000.....	1	1	2	4	3	3	2	1

AVERAGE HEIGHT OF PLANTS (CENTIMETERS)

4,000.....	14	20	23	29	25	27	25	26
5,000.....	12	19	24	23	24	25	24	27
6,000.....	10	20	21	21	19	23	22	26
7,000.....	7	6	20	10	14	21	10	12
8,000.....	1	8	8	8	8	13	16	7
9,000.....	.....	5	5	4	4	5	7	4
10,000.....	.....	0	1	3	3	8	7	1
11,000.....	1	1	2	3	2	4	3	4

DRY MATTER PRODUCED PER GLASS (GRAMS)

4,000.....	0.090	0.136	0.147	0.216	0.197	0.166	0.155	0.196
5,000.....	.072	.123	.125	.131	.131	.120	.112	.202
6,000.....	.028	.131	.145	.113	.097	.145	.137	.154
7,000.....	.026	.018	.139	.046	.090	.085	.050	.078
8,000.....	0	.040	.055	.051	.048	.055	.074	.052
9,000.....	0	.019	.026	.024	.019	.035	.043	.021
10,000.....	0	0	.011	.008	.018	.065	.036	.028
11,000.....	.001	.001	.010	.019	.013	.014	.016	.025

From Table IV it is seen that the number of seeds germinating, the average height of plants, and the dry matter produced all decrease with the increased concentration of the alkali. The plants appear able to endure alkali better with a fair supply of moisture in the soil than where the soil is dry. This may be due to the fact that the soil solution is diluted by the water. Where the soil moisture was as low as 12.5 per cent, growth practically ceased at 7,000 p. p. m. of sodium carbonate, but in the wetter soils there was growth with as high a concentration as 11,000 p. p. m.

RESULTS IN 1913

On account of the inability to use a large number of seeds in glass tumblers, germination tests were made in tin plates in which 100 seeds could be used. An equivalent of 150 gm. of dry soil was placed in each tin plate and the necessary quantity of dry salt added. The salt was well mixed into the soil, which was made up to about 20 per cent of moisture. The seeds were planted and the pans covered with glass to prevent the escape of moisture. The number of seeds germinating was determined every day for three weeks. The results are summarized in Table V.

TABLE V.—*Percentage of germination of seeds of New Zealand wheat which germinated in 21 days in Greenville soil containing various alkali salts. Salts added dry*

Concentration of salt (p. p. m. of dry soil).	Percentage of germination.					
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate.	Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate+1 per cent of calcium sulphate.
None	92	92	92	92	92	92
2,000	65	84	100	89	88	86
4,000	6	92	91	89	86	83
6,000	2	81	69	90	63	47
8,000	0	88	53	91	13	13
10,000	0	99	12	86	8	0
12,000	0	62	14	92	0	0
14,000	0	21	17	85	0	0
16,000	0	7	2	79	0	0
18,000	0	4	0	88	0	0
20,000	0	0	1	83	0	0

On examining Table V it is seen that sodium chlorid was by far the most toxic of the alkali salts and magnesium sulphate the least. The data given can not be taken as final, since all of the salts were not entirely dissolved and white salts could be seen scattered throughout the soil. The low harmfulness of sodium carbonate was probably due in part to the fact that it is not so readily soluble as the other salts when applied dry.

The mixed salts were more harmful than any single salt, with the exception of sodium chlorid, and it is probable that the harmfulness of the mixed salts was due largely to the sodium chlorid.

Since there was such a great difference in the effects of the various salts, a second experiment was made to determine more exactly the critical point of concentration. The results of this test are summarized in Table VI.

TABLE VI.—*Percentage of germination of New Zealand wheat in soil containing alkali salts in different quantities. Salts added dry*

Sodium chlorid.		Sodium carbonate.		Sodium sulphate.		Magnesium sulphate.		Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate.		Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate + 1 per cent of calcium sulphate.	
P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.
	P. ct.		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*
None.	92	None.	92	None.	92	None.	92	None.	92	None.	92
800	81	10,000	81	2,000	80	12,000	86	4,000	77	1,000	77
1,600	82	11,100	64	4,000	83	14,000	79	5,000	78	2,000	79
2,400	76	12,200	66	6,000	85	16,000	75	6,000	54	3,000	79
3,200	50	13,300	32	8,000	79	18,000	82	7,000	51	4,000	79
4,000	13	14,400	50	10,000	69	20,000	81	8,000	76	5,000	73
4,800	6	15,500	36	12,000	43	22,000	78	9,000	19	6,000	75
5,600	7	16,600	38	14,000	20	24,000	87	10,000	12	7,000	68
6,400	0	17,700	23	16,000	16	26,000	66	11,000	6	8,000	46
7,200	0	18,800	13	18,000	3	28,000	56	12,000	10	9,000	38
8,000	0	19,900	1	20,000	0	30,000	57	13,000	1	10,000	13

An examination of Table VI, in agreement with Table V, shows the germination to be greatly reduced by sodium chlorid in concentrations above 3,000 p. p. m., while it ceases entirely at about 6,000 p. p. m. With sodium carbonate a large reduction in germination occurred at about 10,000 p. p. m., but a few plants survived at about 20,000 p. p. m. The sodium sulphate showed about the same results as the sodium carbonate, while the magnesium sulphate gave over a 50 per cent germination at a concentration of 30,000 p. p. m. In the mixed salts the gypsum (calcium sulphate) did not have any great effect, possibly owing to the slowness with which gypsum dissolves.

On comparing the data in Tables V and VI with those reported in Table I and also others given later in the paper, where the salts were first dissolved and added in solution, it will be found that the salts were more toxic when added in solution than when mixed with the dry soil. This may be due to the slow solution and diffusion of the salt when added dry, which probably helps to explain the common observation that crops can sometimes be made to grow in a soil the analysis of which shows a very high total alkali content. It also explains why it is that crops growing on alkali land may look healthy and be growing vigorously until irrigated, when they are immediately killed.

In order to determine more exactly the effect of soil moisture on the toxicity of alkali salts, sand was placed in tin plates, as previously

described. To this sand salts were added in solution with the quantity of water necessary to bring the sand to the desired moisture content. Twenty-five kernels of Turkey Red wheat were planted in each pan, which was then covered with window glass to retain the moisture. Any loss in moisture was made up from time to time. The percentage of germination at the end of three weeks is given in Table VII.

TABLE VII.—*Percentage of germination at the end of three weeks of the seeds of Turkey Red wheat in sand with different quantities of moisture and alkali salts. Salts added in solution*

Salt and concentration (p. p. m. of dry soil).	Percentage of water in sand.				
	12	15	18	21	24
Sodium chlorid:					
None	75	80	84	84	78
800	92	80	72	88	80
1,800	48	80	88	76	48
2,400	28	60	88	80	60
2,900	4	24	68	64	44
3,600	0	0	84	12	16
4,000	0	0	36	0	8
4,500	0	0	6	0	0
5,700	0	0	4	0	0
6,000	0	0	0	0	0
Sodium carbonate:					
None	75	80	84	84	78
1,200	72	68	72	84	76
1,600	44	56	56	60	64
2,000	28	36	32	44	56
2,700	8	4	4	24	24
3,300	0	0	0	4	4
4,000	0	0	0	0	0
4,700	0	0	0	0	0
Sodium sulphate:					
None	75	80	84	84	78
2,000	88	88	92	96	88
4,000	36	72	80	92	68
6,000	12	60	72	72	72
8,000	8	4	20	44	64
10,000	0	0	28	36	36
12,000	0	0	0	12	20
14,000	0	0	0	0	4
16,000	0	0	0	0	4
18,000	0	0	0	0	0
Magnesium sulphate:					
None	75	80	84	84	78
12,000	20	24	40	28	56
14,000	16	12	48	48	60
16,000	12	16	48	52	48
18,000	4	8	20	44	40
20,000	0	4	8	16	48
22,000	0	0	0	12	12
24,000	0	0	0	12	12
26,000	0	0	0	0	4
28,000	0	0	0	0	0

From Table VII it will be seen that germination was first retarded by the salts when the soils contained but a small amount of moisture. With most of the salts the highest germination was in the wettest sand, while with sodium chlorid the intermediate moisture gave the highest germination.

It will be noted that in the sand sodium carbonate was more toxic than sodium chlorid. This same relation is also reported later in this paper with sand, although in all the tests with loam sodium chlorid was more toxic than sodium carbonate. A comparison of the limits of growth in sand with those already reported for loam brings out the fact that germination is reduced by a much lower concentration in sand than in loam. This is also brought out clearly in results reported later.

OUTLINE OF LATER WORK

GENERAL METHODS OF EXPERIMENTATION

A number of experiments were conducted in glass tumblers in which an equivalent of 200 gm. of dry soil was placed. Salts were added to the soil as follows: A stock solution of each salt was made up, containing an equivalent of 10 per cent of the anhydrous salt. The necessary quantity of the stock solution was then added to sufficient distilled water to make the soil up to 20 per cent water on the dry basis. The water containing the solution was thoroughly mixed with the soil on oilcloth and the whole placed in the glass. This method insured an even distribution of the salt through the soil.

In all cases the soil was made up to 20 per cent with moisture. This was about the optimum amount for plant growth. Ten seeds were planted in each glass to a depth of ½ inch from the surface. After the seeds were planted the glass tumblers were covered with panes of window glass until the plants were up. This prevented evaporation and enabled the seeds to germinate with an even soil-moisture content.

Counts were made of the number of plants up each day, which made possible a determination of the relative time required for germination in the different treatments. The original moisture content was maintained by adding the necessary quantity of water every day or two. The plants were allowed to grow for two or three weeks, when they were harvested and measured and the dry weights determined.

The data obtained for each glass therefore included (1) the percentage of germination, (2) the average time required for germination, (3) the average height of plants, (4) the average number of leaves, and (5) the dry matter produced.

In each test there were 15 glasses for each concentration of salts, and there were 10 concentrations. In addition, there were four check glasses to which no salt was added. This made 154 glasses for each test. In the series there were 24 tests, which gave a total of 3,696 glasses.

Five determinations were made of the plants in each glass, making about 18,450 separate determinations. This number was reduced somewhat by the fact that plants did not germinate in all the glasses, owing to the high salt content. With this great number of results it is impracticable to give all the data in detail; hence, only summaries will be presented.

COMBINATION OF SALTS

In each test containing 15 glasses three different salts were used. The glasses were arranged in the triangular diagram used in expressing

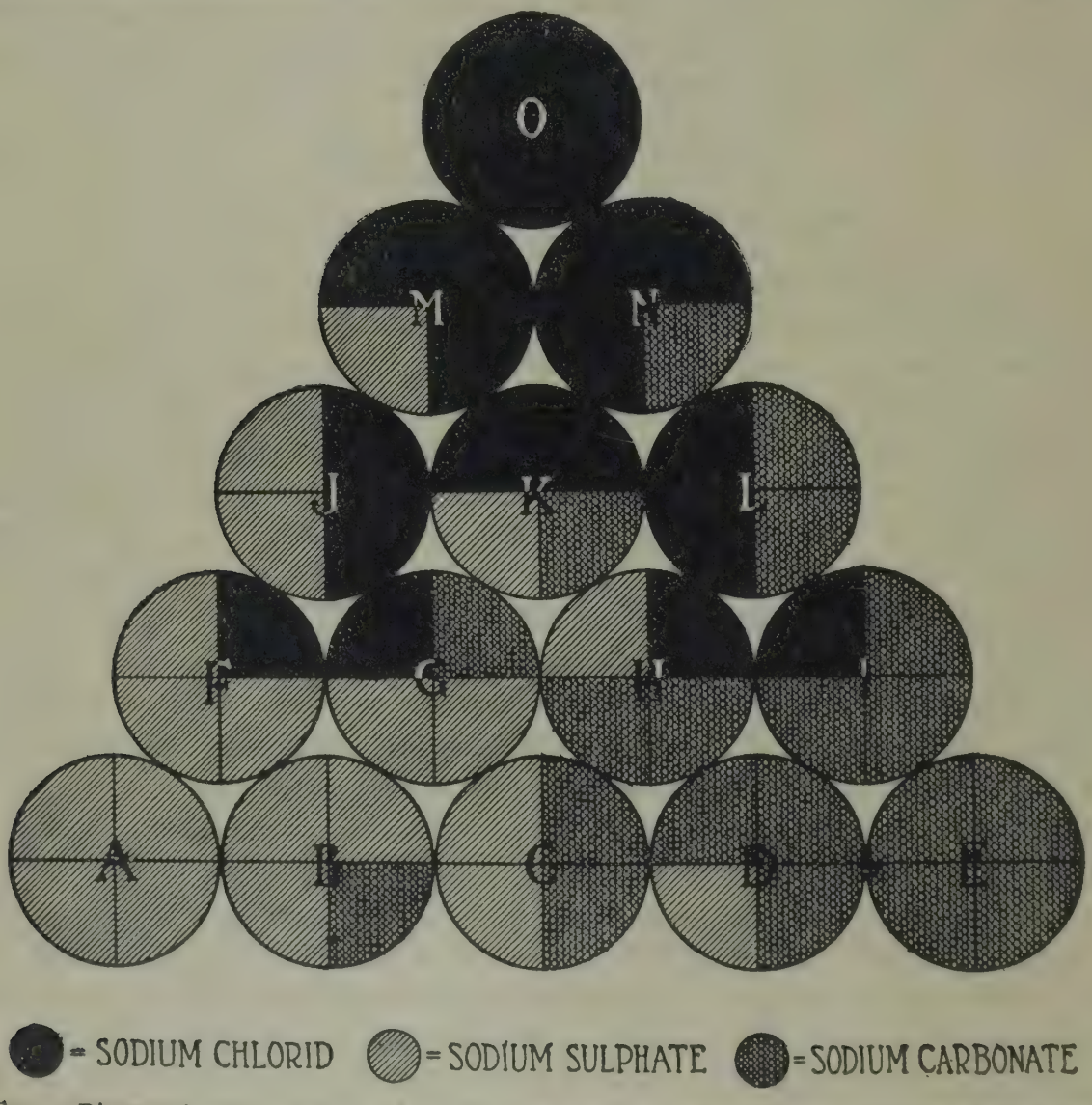

FIG. 1.—Diagram showing percentage of salts, mixtures, and their position in the diagrams of experimental sets. The arrangement of salts shown here is that in figure 2, page 14. The same positions but with different salts apply to figures 2 to 25.

three variables. This arrangement is shown in figure 1, the salts in this case being sodium sulphate, sodium carbonate, and sodium chlorid. All 15 glasses contain the same total concentration of salts—for example, in figure 1 the concentration is 1,000 parts of salt per million parts of dry soil.

The glasses on the corners of the diagram which are marked "A," "E," and "O" contained 100 per cent of the single salts. The other glasses

along the sides contained a mixture of two salts, while the glasses in the center contained all three salts in the proportions indicated.

It will be noted that the top glass (O) contained 100 per cent of sodium chlorid, the second row, with glasses M and N, 75 per cent of sodium chlorid, the third row, with glasses J, K, and L, 50 per cent of sodium chlorid, the fourth row, with glasses F, G, H, and I, 25 per cent of sodium chlorid, while the bottom row contained no sodium chlorid. The same order is followed with each of the other salts. Thus, there are glasses with each of the single salts, others with two salts in various combinations, and still others with all three salts in different proportions. From this arrangement it is possible to determine the effects of the single salts as well as the various combinations of salts.

In order to find the effects of the concentration of salts, 10 different concentrations were tried for each three salts. These varied from 1,000 to 10,000 p. p. m. of salt based on the dry soil. The combination of salts, as well as the soils and crops, are given in Table VIII.

TABLE VIII.—*Combinations of salts, soils, and crops used in concentration experiments*

Trial No.	Combination of salts.	Soil.	Crop.
1	Sodium chlorid, sodium sulphate, sodium carbonate.	Greenville loam	New Zealand wheat.
2	Potassium chlorid, calcium chlorid, magnesium chlorid.	do	Do.
3	Sodium nitrate, potassium nitrate, magnesium nitrate.	do	Do.
4	Sodium sulphate, potassium sulphate, magnesium sulphate.	do	Do.
5	Potassium carbonate, sodium carbonate, ammonium carbonate $(NH_4)_2CO_3$.[a]	do	Do.
6	Sodium chlorid, sodium sulphate, sodium carbonate.	Coarse sand	Do.
7	Potassium chlorid, calcium chlorid, magnesium chlorid.	do	Do.
8	Sodium nitrate, potassium nitrate, magnesium nitrate.	do	Do.
9	Sodium sulphate, potassium sulphate, magnesium sulphate.	do	Do.
10	Potassium carbonate, sodium carbonate, ammonium carbonate.	do	Do.
11	Sodium chlorid, sodium sulphate, sodium carbonate.	College loam	Do.
12	Potassium chlorid, calcium chlorid, magnesium chlorid.	do	Do.
13	Sodium nitrate, potassium nitrate, magnesium nitrate.	do	Do.
14	Sodium sulphate, potassium sulphate, magnesium sulphate.	do	Do.
15	Potassium carbonate, sodium carbonate, ammonium carbonate.	do	Do.
16	Sodium chlorid, sodium sulphate, sodium carbonate.	Greenville loam	Chevalier barley.
17	Do	do	White flint corn.
18	Do	do	Danish oats.
19	Do	do	Sugar beets.
20	Do	do	Alfalfa.
21	Do	do	Canada field peas.
22	Do	Distilled water	New Zealand wheat.
23	Potassium chlorid, calcium chlorid, magnesium chlorid.	do	Do.
24	Sodium nitrate, potassium nitrate, magnesium nitrate.	do	Do.

[a] The ammonium carbonate used has the formula $(NH_4)_2CO_3(NH_4)_2CO_2NH_2$, but the simpler formula, $(NH_4)_2CO_3$, is used for convenience.

DESCRIPTION OF SOILS

The following analyses were made by members of the Utah Station staff from soils taken from the same fields as the soils used in the experiments. While the analyses are not of the exact soils used, they will be useful, since the soils in these fields are very uniform. See Tables IX and X.

TABLE IX.—*Chemical analysis of soils used (strong hydrochloric-acid digestion)* [1]

Constituent.	Greenville soil.	College loam.	Sand.
	Per cent.	*Per cent.*	*Per cent.*
Insoluble residue	42.18	66.69	51.06
Potash (K_2O)	.67	.55	.15
Soda (Na_2O)	.35	.49	.21
Lime (CaO_2)	16.88	7.41	17.43
Magnesia (MgO)	6.10	4.15	5.63
Iron oxid (Fe_2O_3)	3.03	2.93	.86
Alumina (Al_2O_3)	5.64	3.49	1.25
Phosphoric acid (P_2O_5)	.41	.25	.14
Sulphuric acid (H_2SO_4)	.....	.07	.03
Carbon dioxid (CO_2)	19.83	7.62	20.73
Humus	.53	2.18	.23
Total nitrogen	.14	.15	.02

TABLE X.—*Physical analysis of soils used (determined with Yoder elutriator)*

[1] Constituent.	Greenville soil.	College loam.	Sand.
	Per cent.	*Per cent.*	*Per cent.*
Coarse sand (above 1 mm.)	9.84	17.69	70.49
Fine sand (1 to 0.03 mm.)	30.04	37.39	20.75
Coarse silt (0.03 to 0.01 mm.)	32.25	15.19	3.32
Medium silt (0.01 to 0.003 mm.)	12.30	10.36	1.54
Fine silt (0.003 to 0.001 mm.)	6.25	10.32	.81
Clay (below 0.001 mm.)	7.62	9.03	2.16
Real specific gravity	2.67	2.64	2.81
Apparent specific gravity	1.23	1.32	1.32

[1] For methods followed, *see* Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem., Bul. 107 (rev.), 272 p., 1908.

DETAILS OF GERMINATION OF PLANTS AND DRY MATTER PRODUCED

GREENVILLE SOIL

In accordance with the outline already given, five tests were made with Greenville soil, three different salts being used in each test. The arrangement of glasses, the number of seeds germinated, and the dry matter produced in each glass are given in figures 2, 3, 4, 5, and 6. The name of the salt is given at the corner of each triangle. The combination of

these salts can readily be determined by consulting figure 1. The number at the bottom of each triangle refers to the concentration of soluble salts in all the glasses of that triangle expressed in parts of anhydrous salt per million parts of dry soil.

An examination of figure 2 shows that some seeds germinated in all glasses up to a concentration of 4,000 p. p. m., but that at 5,000 p. p. m. there was no germination in the glass having all sodium chlorid, and only germination in one of the glasses with three-fourths sodium chlorid. In the part of the triangle toward the sodium chlorid the germination gradually decreased as the concentration increased. The sodium carbonate and sodium sulphate showed almost a complete germination up to 10,000 p. p. m., or 1 per cent of salt.

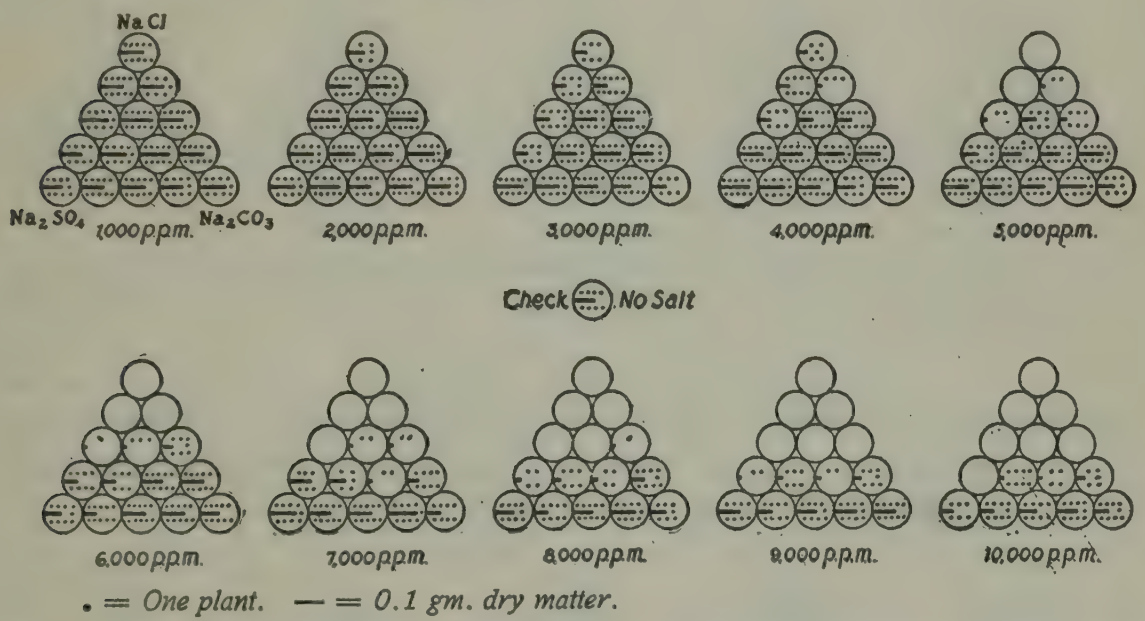

FIG. 2.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

The greater toxicity of sodium chlorid as compared with sodium carbonate was somewhat of a surprise, since most of the literature on alkali considers sodium carbonate, or black alkali, as being by far the most harmful of the alkali salts. The results given here agree with those found in the experiments of 1912 and 1913 and are also borne out by the results shown in figures 7, 12, 17, 18, 19, 20, 21, and 22, where different crops are compared.

In the glasses that received sodium carbonate the surface was black with dissolved humus and was somewhat crusted, showing that the physical condition had been injured. Notwithstanding this fact, seeds germinated in the soil and the plants grew for three weeks with no great injury except a slight blackening of plants at the surface of the soil with higher concentrations.

Figure 3 shows results for the chlorids of potassium, calcium, and magnesium. These chlorids are not as toxic as the chlorid of sodium,

but they are all more toxic than the sodium sulphate and sodium carbonate. Magnesium chlorid seemed to be the least toxic of the chlorids that were tested. Germination in all of them fell off rapidly above 4,000 p. p. m.

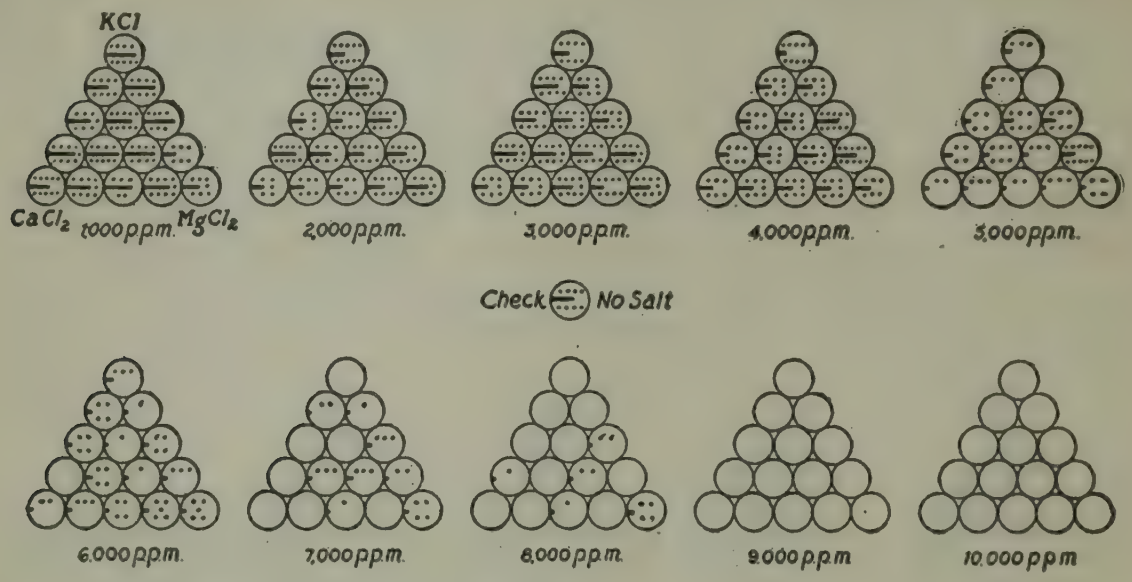

FIG. 3.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

In figure 4 the nitrates of sodium, potassium, and magnesium are compared and the sodium found to be slightly more toxic than the others. The nitrates appear on the whole to be somewhat less toxic than the chlorids, but more so than the sulphates or carbonates.

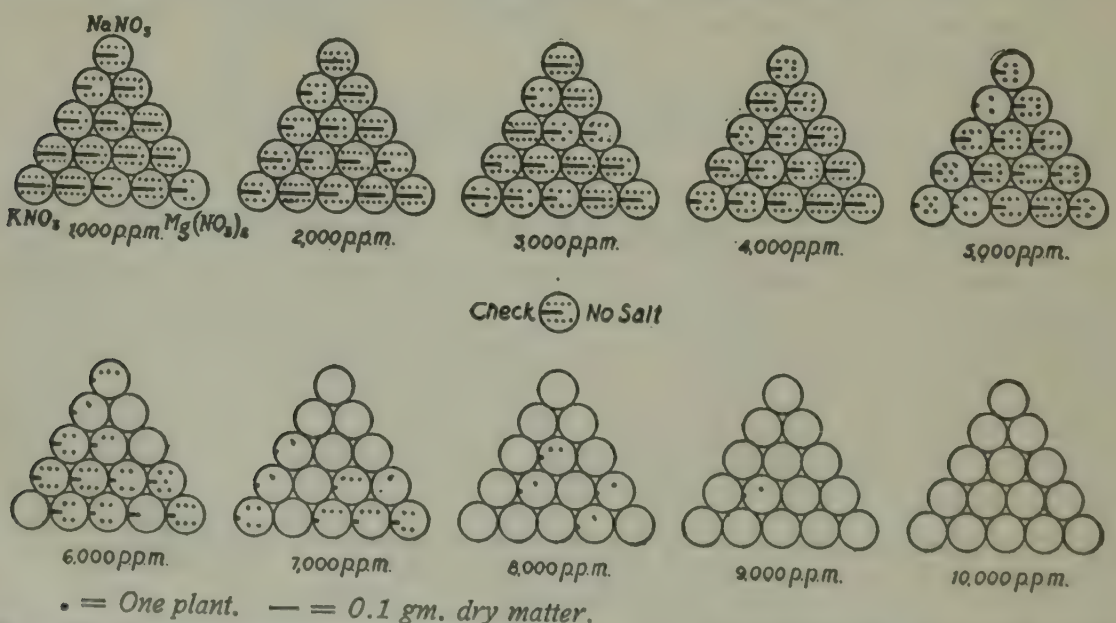

FIG. 4.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

The results for the sulphates of sodium, potassium, and magnesium are given in figure 5. There was practically complete germination with all of the sulphates up to a concentration of 1 per cent; hence, but little difference in the three salts can be seen.

With the carbonates shown in figure 6 there is a marked falling off with the ammonium carbonate above 5,000 p. p. m. With the others there is a good germination up to 10,000 p. p. m., similar to the results shown in figure 2. The formula given by the manufacturers of the ammonium

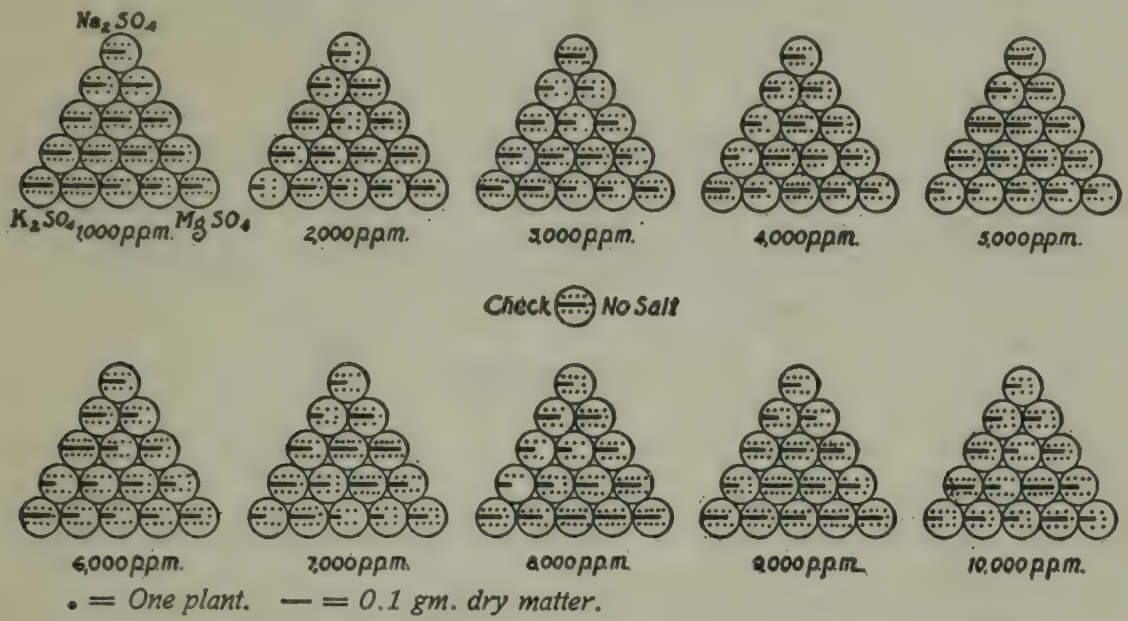

FIG. 5.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

carbonate was $(NH_4)_2CO_3(NH_4)CO_2NH_2$ instead of the shorter formula, $(NH_4)_2CO_3$, given on the figures.

It is probable that the toxicity of the ammonium carbonate was due, in part at least, to the free ammonia that was constantly being given off

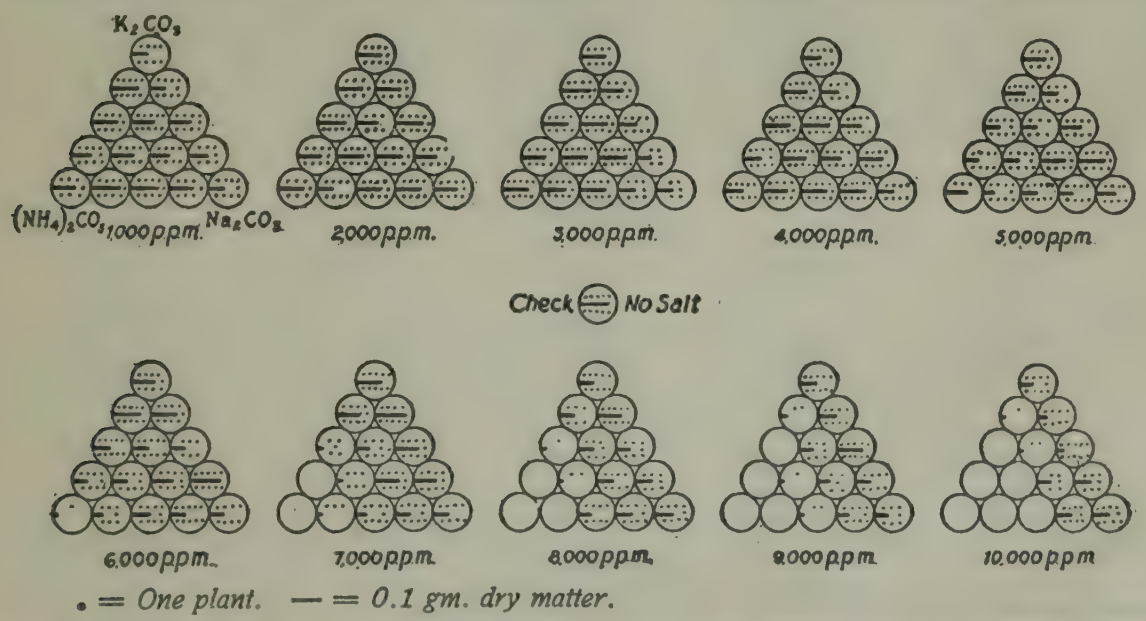

FIG. 6.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

by this unstable compound rather than to the CO_3 part of the compound. It is a well-known fact that protoplasm is very sensitive to the action of free ammonia.

SAND

Five sets of tests were conducted with wheat growing in sand similar to those with the Greenville soil.

In figure 7 the results for sodium chlorid, sodium sulphate, and sodium carbonate are given. The noticeable thing about these results, as well

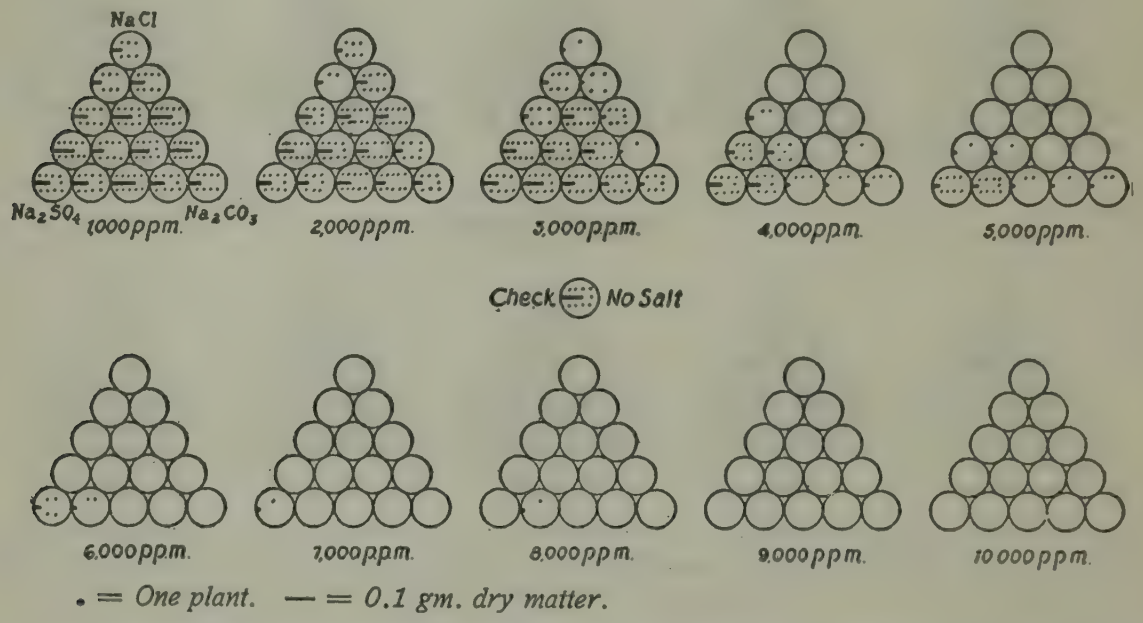

FIG. 7.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

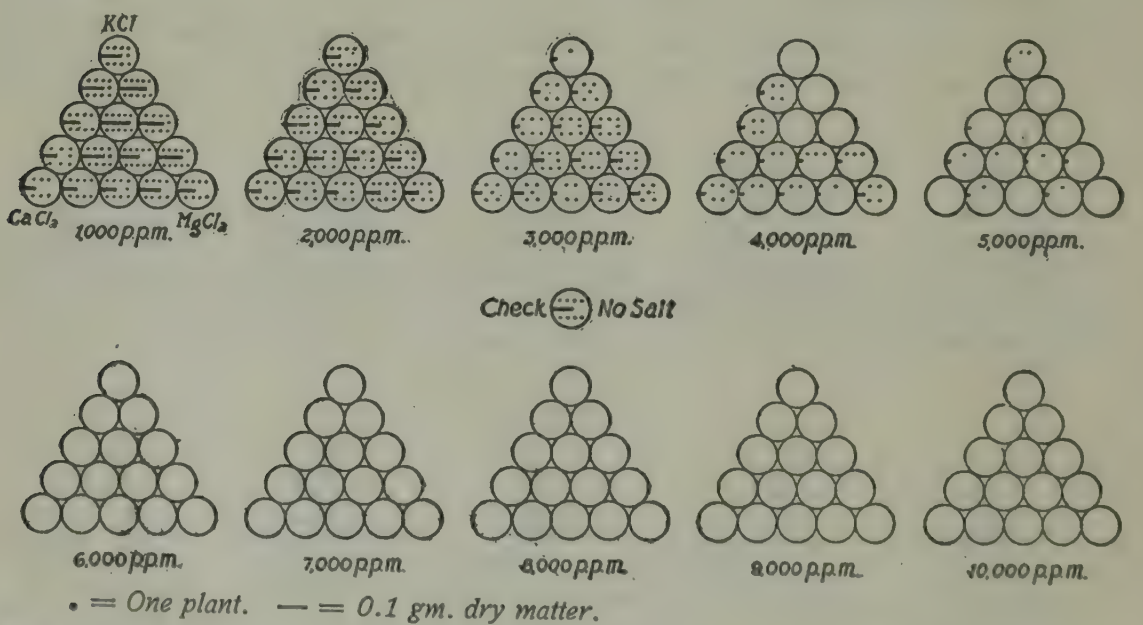

FIG. 8.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

as all those for sand, is that only about half as much salt is required to stop growth in sand as in either the Greenville soil or the College loam.

The same general relations between the salts are shown here as in the Greenville soil, except that in the sand sodium carbonate is propor-

tionately more toxic than in the other soils. This is exactly the same result that was obtained in 1913 in the experiments already described. In sand the carbonates seem to be nearly as toxic as the chlorids, while in the other soil they are very much less injurious.

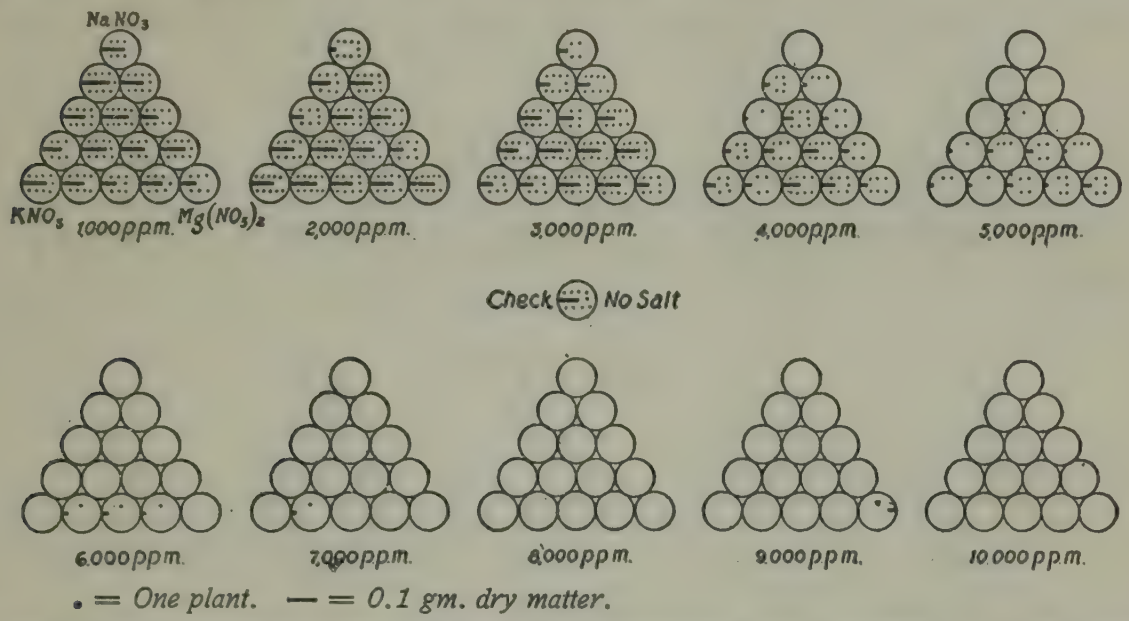

FIG. 9.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

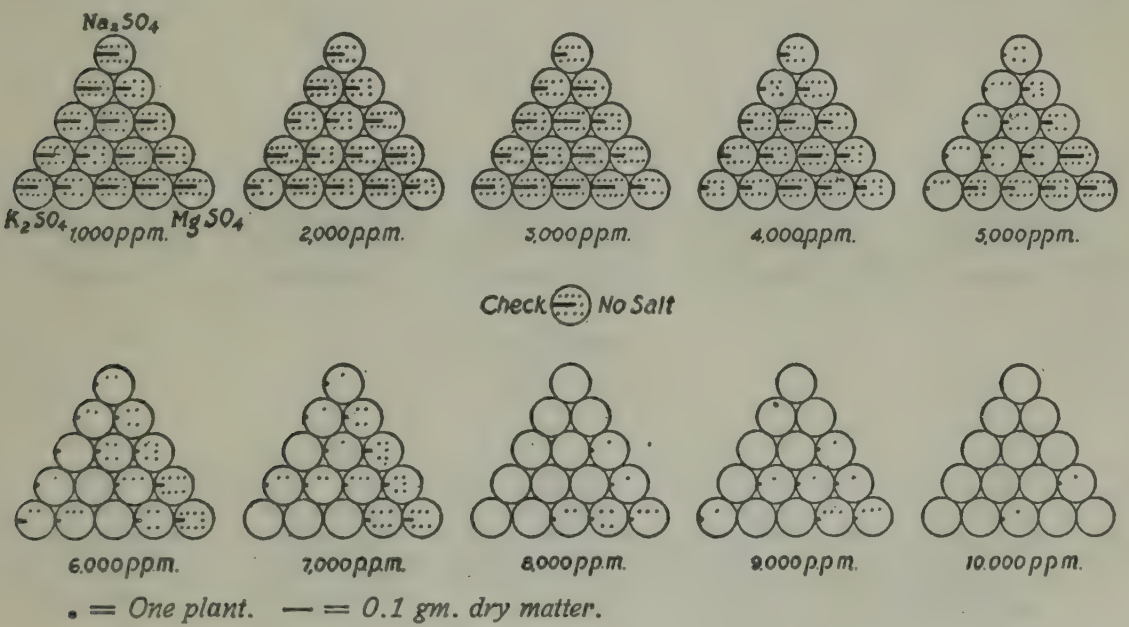

FIG. 10.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

Figure 8 shows the same relationship between the chlorids as was brought out in figure 3. It also shows that these salts are in injurious lower concentrations in sand than in other soils.

The nitrates are shown in figure 9 to be slightly less injurious than the chlorids in figure 8. The sodium salt is again shown to be more injurious than the others.

In sand the limit of growth in the presence of sulphates is shown by figure 10 to be less than 10,000 p. p. m., while in the loam growth was scarcely retarded at this concentration. Plants seem able to resist decidedly more magnesium sulphate than either potassium sulphate or

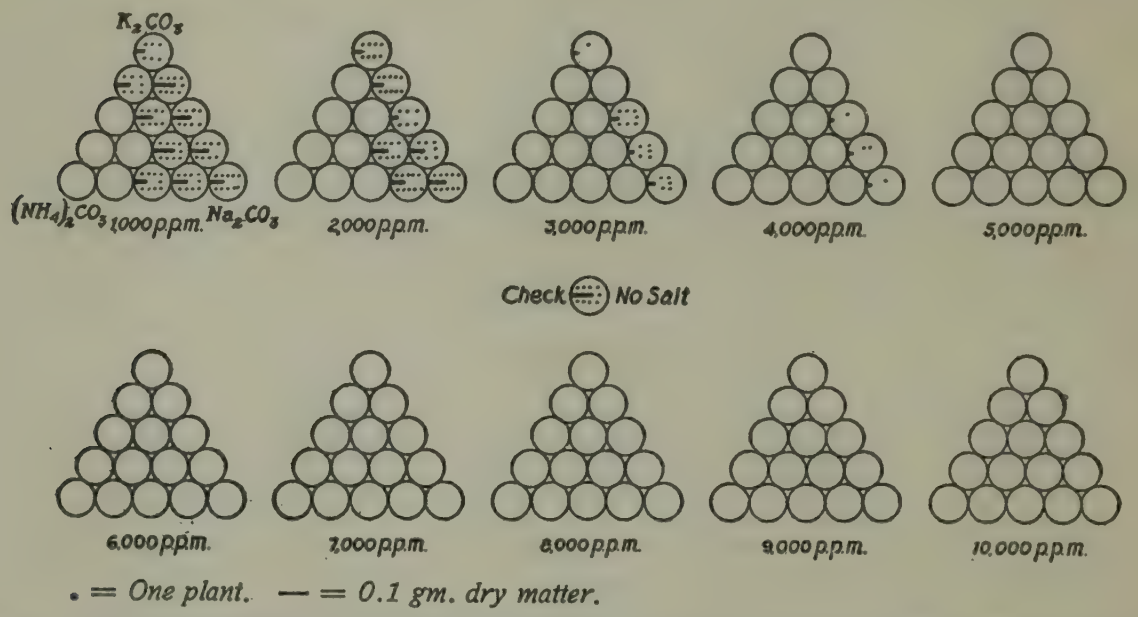

FIG. 11.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

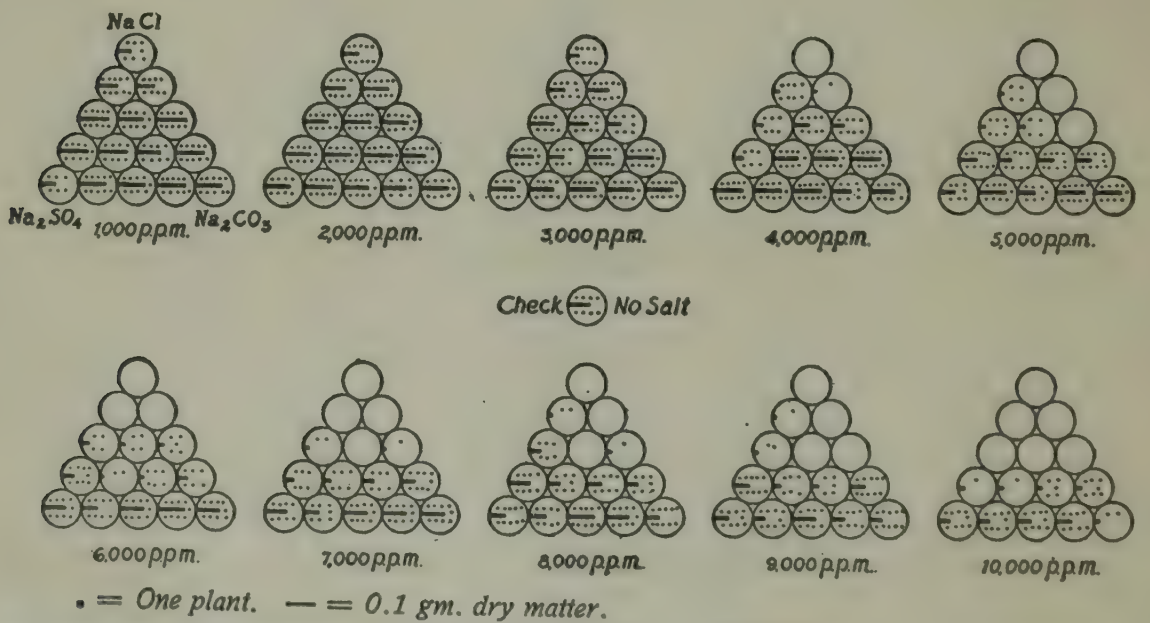

FIG. 12.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

sodium sulphate. This is in accord with the earlier results found in 1912 and 1913.

Figure 11 shows that there was no germination whatever in sand where even as little as 1,000 p. p. m. of ammonium sulphate were found. With

any of the carbonates there was no germination for concentrations above 4,000 p. p. m.

COLLEGE LOAM

The same number of tests, using the same kinds of salts and seeds were conducted in College loam as in Greenville soil and sand. The

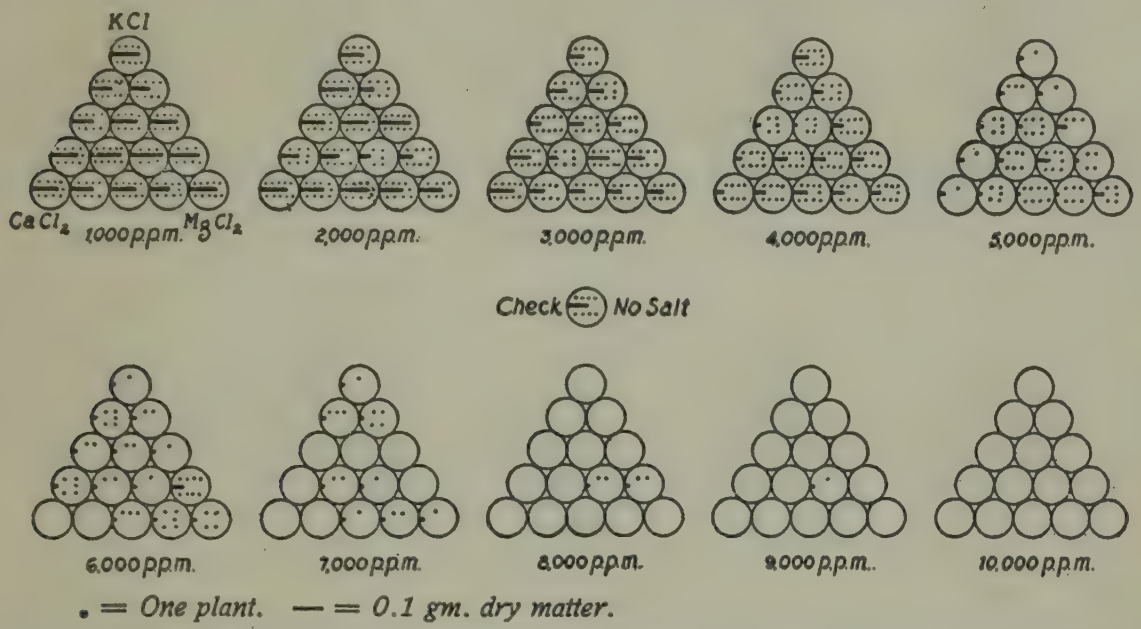

FIG. 13.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

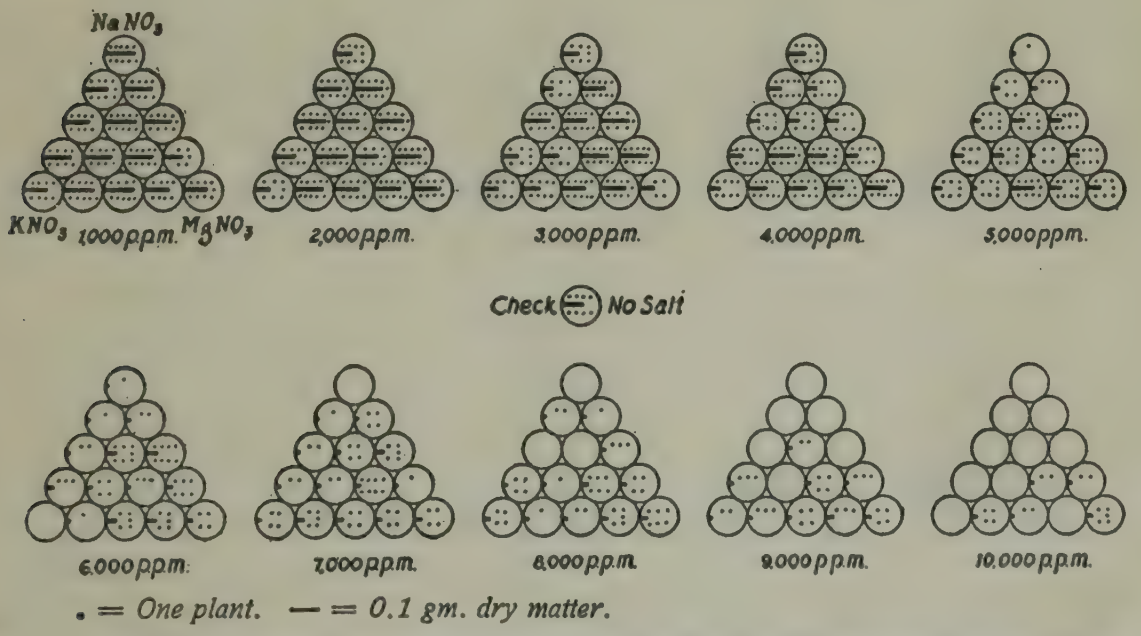

FIG. 14.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

results are shown in figures 12, 13, 14, 15, and 16. These results agree so completely with those found for the Greenville soil that individual comment seems unnecessary.

COMPARISON OF CROPS

In the management of alkali land it is important to know the relative resistances of various crops. Farmers who have been accustomed to deal with alkali are well aware that certain crops can be made to grow where others would be a complete failure.

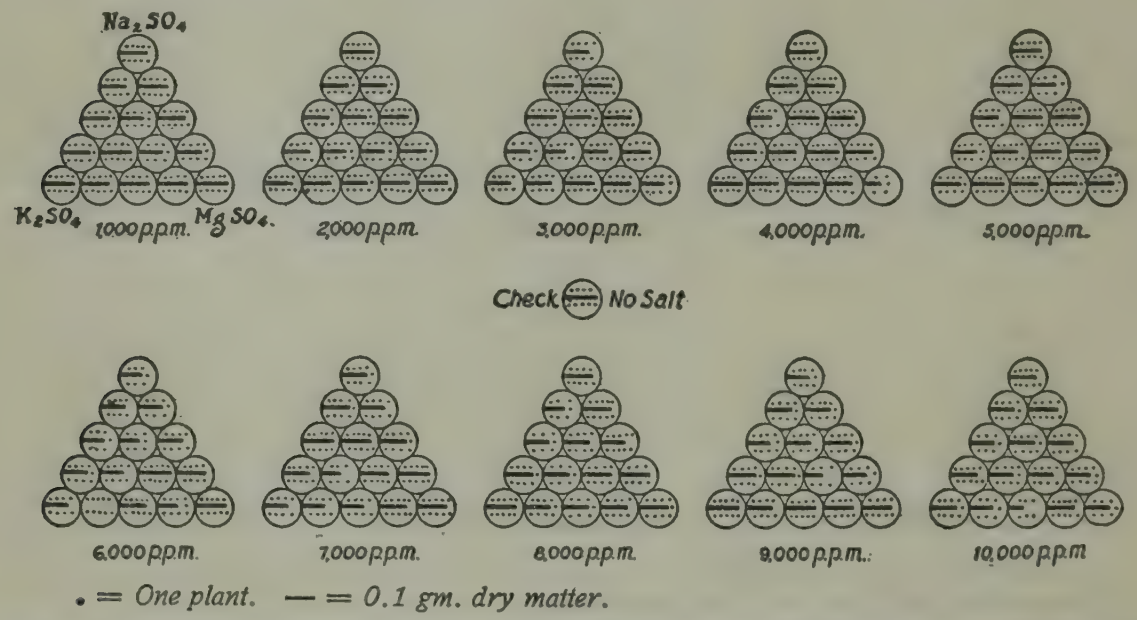

FIG. 15.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

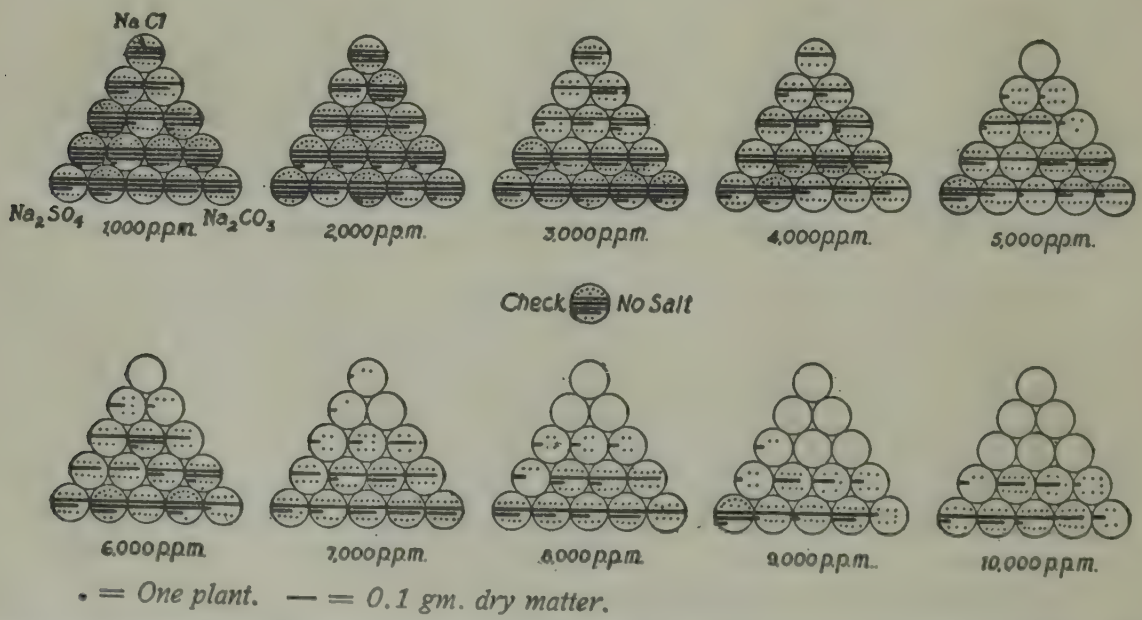

FIG. 16.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

A number of the common field crops were tested in the manner already described. Greenville soil was placed in glass tumblers and sodium chlorid, sodium sulphate, and sodium carbonate added in the same combinations and concentrations previously used. Ten seeds were

planted in each glass. The crops compared were wheat (*Triticum* spp.), barley (*Hordeum* spp.), oats (*Avena sativa*), corn (*Zea mays*), alfalfa (*Medicago sativa*), sugar beets (*Beta vulgaris*), and Canada field peas (*Pisum arvense*). The results for wheat have already been shown in

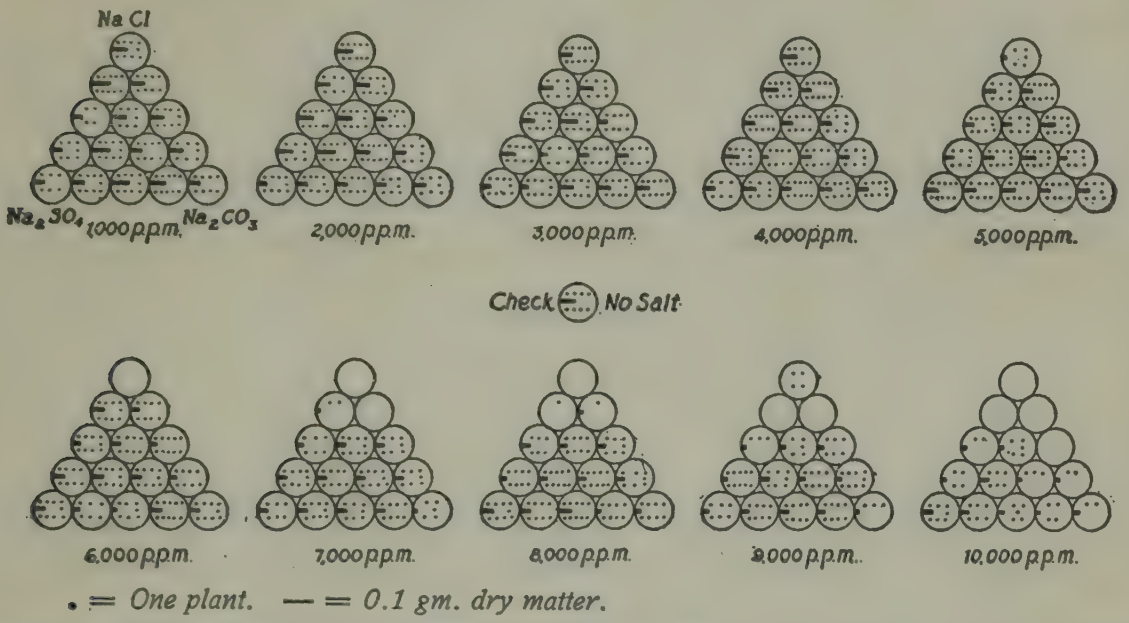

FIG. 17.—Diagram showing the number of barley plants up and dry matter produced in 24 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

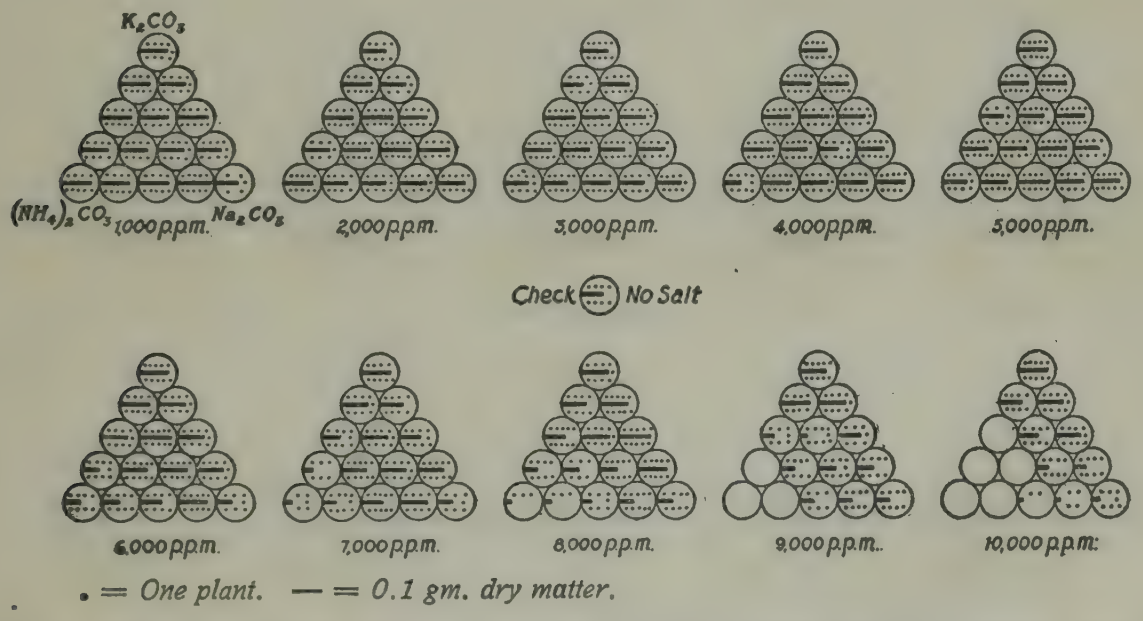

FIG. 18.—Diagram showing the number of corn plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

figure 2, while those for the other crops will be found in figures 17, 18, 19, 20, 21, and 22.

An examination of these diagrams shows that the relation between the salts, pointed out in connection with wheat, holds for the other crops.

According to the resistance of their seedlings to alkali, the crops fall into the following order: (1) Barley, (2) oats, (3) corn, (4) wheat, (5)

sugar beets, (6) alfalfa, and (7) Canada field peas. It may be that after the crops get a good start their resistance would not be in just this order; but in the percentage of seeds germinated this order seems to hold. Barley was able to withstand about twice as much alkali as field peas.

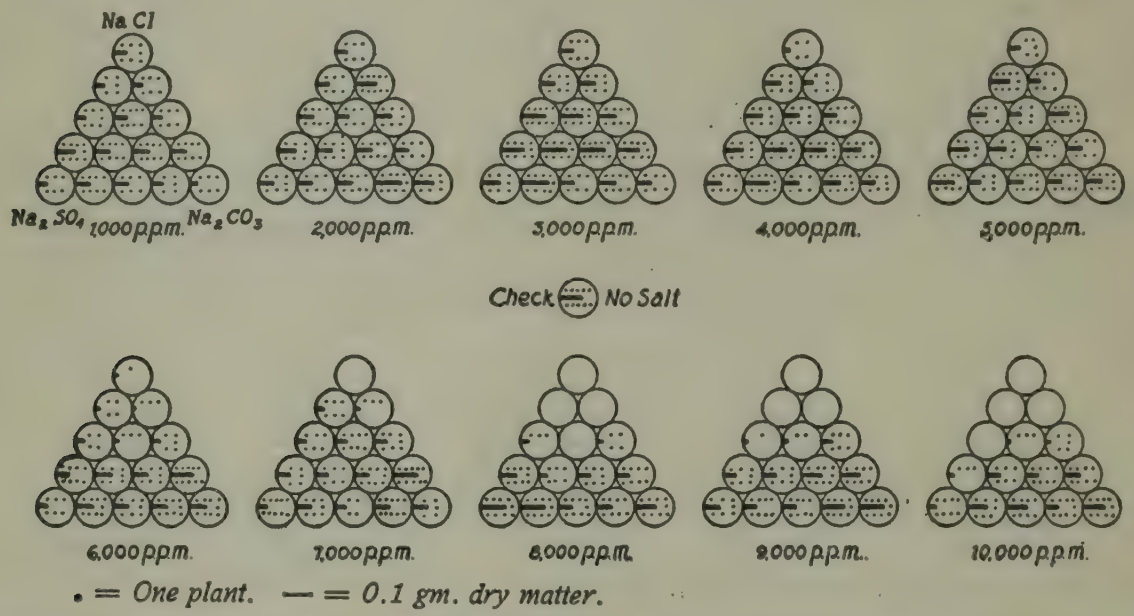

FIG. 19.—Diagram showing the number of oat plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

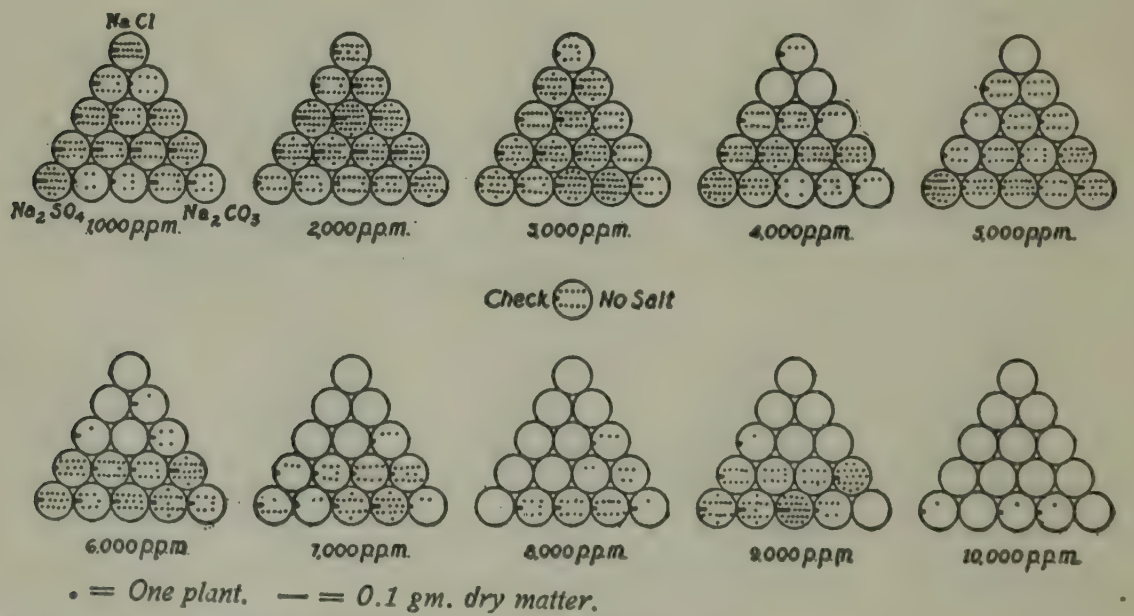

FIG. 20.—Diagram showing the number of sugar-beet plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

SOLUTION CULTURES

In order to compare the effect of salts in solution cultures with the same salts in soils, a number of tests were made with seedlings growing in distilled water to which various salts had been added. Glass tumblers were filled with water containing the proper quantity of the desired

solution. The glasses were then covered with paraffined paper which was bent over the edges and held in place by rubber bands. New Zealand wheat was germinated between moist filter papers until its roots were about half an inch long, when 10 seedlings to each glass were placed in

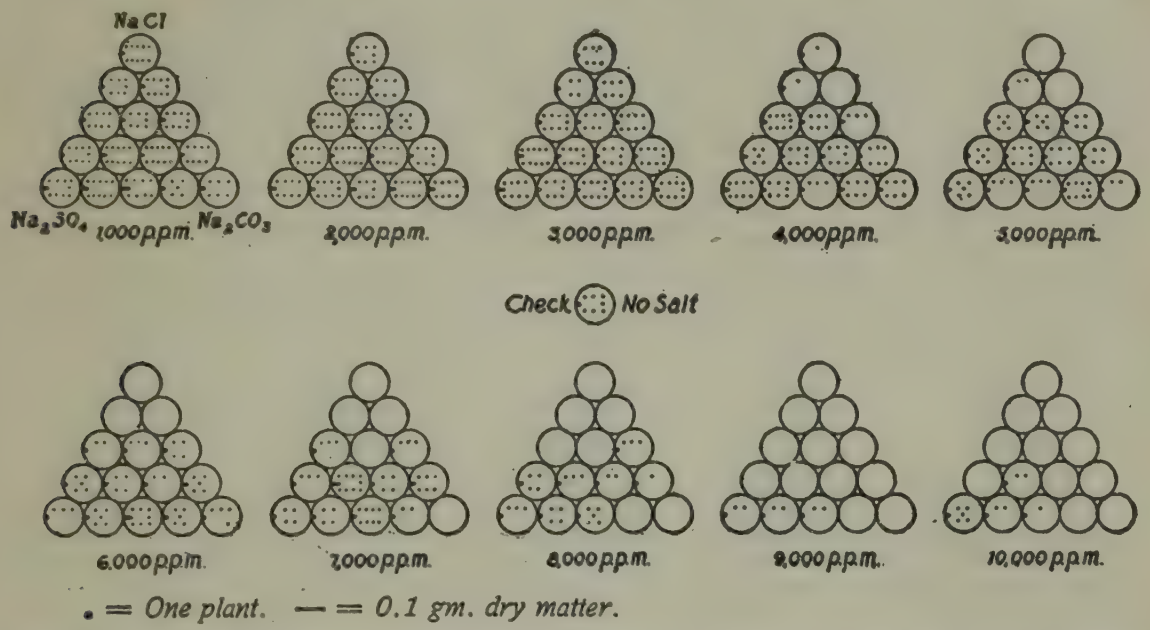

FIG. 21.—Diagram showing the number of alfalfa plants up and dry matter produced in 21 days on College loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

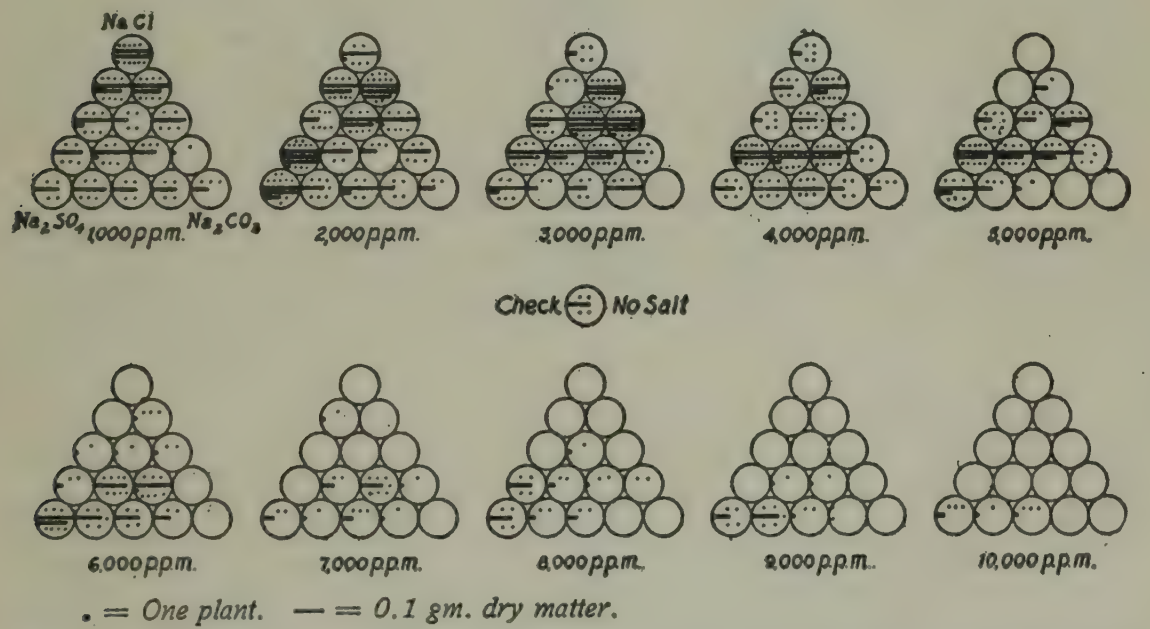

FIG. 22.—Diagram showing the number of Canada field-pea plants up and dry matter produced in 21 days on Greenville loam with sodium chlorid, sodium sulphate, and sodium carbonate in different combinations and concentrations.

holes in the paraffined paper, so that their roots grew down into the solutions.

The loss of water due to transpiration was made up every day or two.

The glasses were arranged in the triangular diagram as in the experiments with soils, which have already been discussed. In each test the

concentrations ranged from 1,000 parts of anhydrous salt for each 1,000,000 parts of water up to 10,000 p. p. m. of salt. The seedlings were allowed to grow 21 days before being harvested. At harvest the following determinations were made of the plants in each glass: (1) Plants still alive, (2) average height of plants, (3) average length of roots, (4) average number of leaves per plant, (5) dry weight of tops, (6) dry weight of roots, (7) ratio of length of tops to length of roots, (8) ratio of weight of tops to weight of roots.

In the first test sodium chlorid, sodium carbonate, and sodium sulphate, were used; in the second, potassium chlorid, calcium chlorid, and magnesium chlorid; and in the third, sodium nitrate, potassium nitrate, and magnesium nitrate. Figures 23, 24, and 25 show in detail the number of

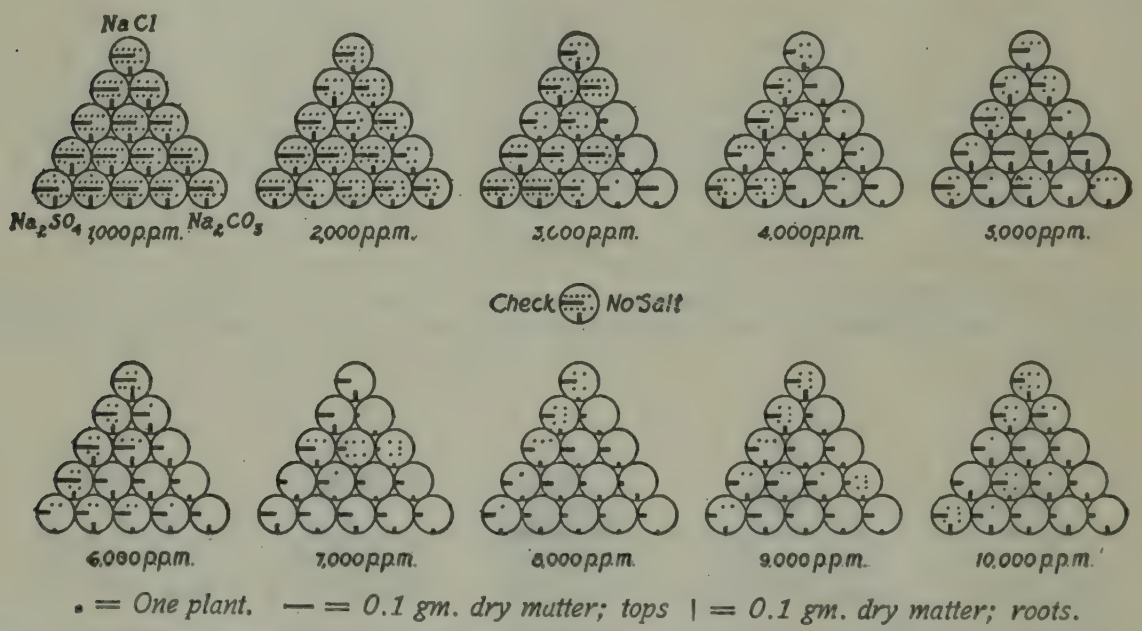

FIG. 23.—Diagram showing the number of seedlings alive and dry matter produced in tops and roots in 21 days with solutions of sodium chlorid, sodium sulphate, and sodium carbonate in different combinations and concentrations.

plants alive at the end of three weeks, as well as the weight of tops and roots in each glass.

An examination of the figures shows a gradual decrease in growth as the concentration of salts increased. Plants were able to endure much stronger chlorids and nitrates in solution culture than in the soil, while the carbonate retarded growth more in the solution than in the loam, but not as much as in the sand. The plants growing in the distilled water without any salts had no food except that stored in the seed and that dissolved from the glass, and, as a result, they produced less growth than plants growing in the dilute solutions.

The results showing the effect of concentration of the various salts are summarized in Table XI. Each figure represents the average of nine different salts of a given concentration. An examination of the table shows that the number of plants alive at the end of three weeks

decreased as the concentration of the solution increased, there being an average of 9.7 plants to each glass alive where no salt was added to the culture, but only 3.8 plants alive with 10,000 p. p. m. of salt.

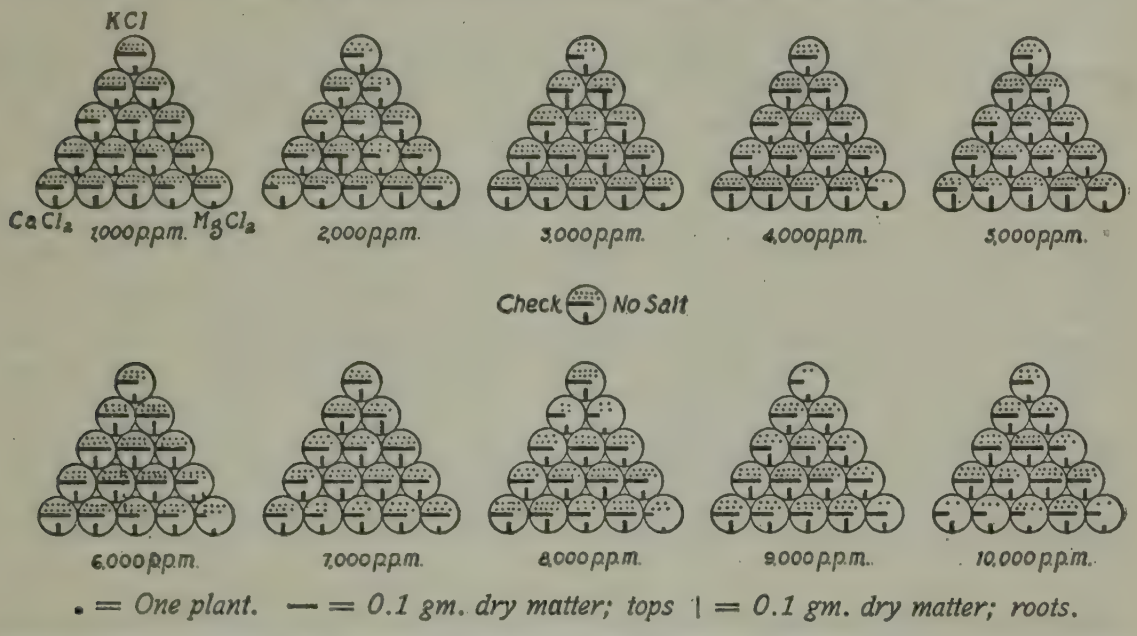

FIG. 24.—Diagram showing the number of wheat seedlings alive and dry matter produced in tops and roots in 21 days with solutions of potassium chlorid, calcium chlorid, and magnesium chlorid in different combinations and concentrations.

There was a corresponding decrease in number of leaves per plant, height of plants, length of roots, weight of tops, and weight of roots as the concentration of salts increased. The weight of roots, however, was not so much affected as some of the other results. In the cultures in

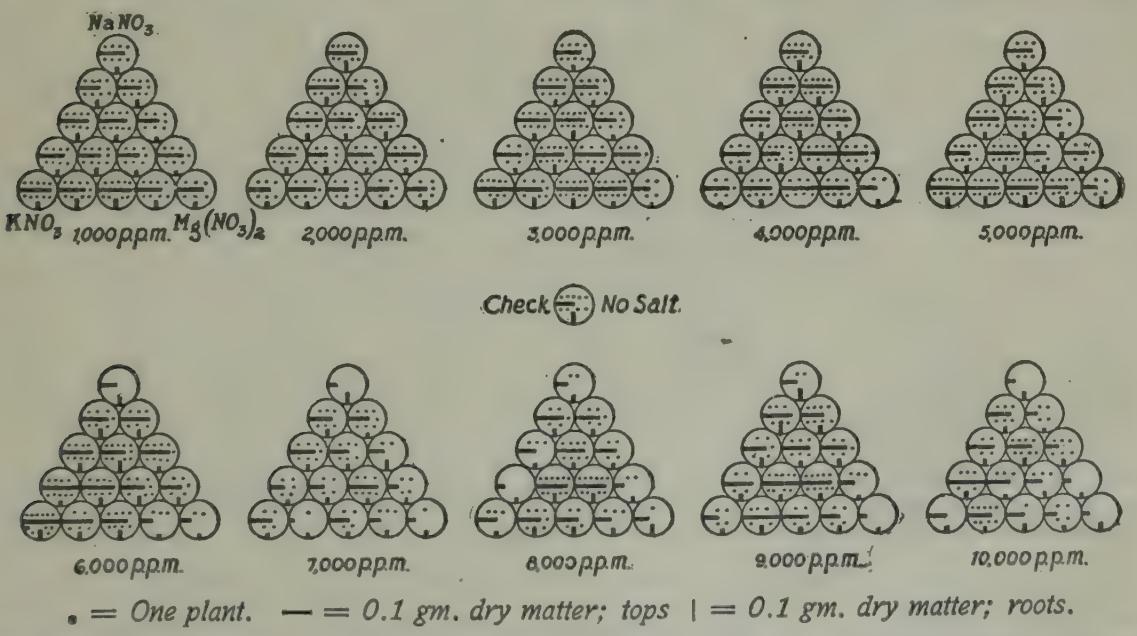

FIG. 25.—Diagram showing the number of wheat seedlings alive and dry matter produced in tops and roots in 21 days with solutions of sodium nitrate, potassium nitrate, and magnesium nitrate in different combinations and concentrations.

which no salts were added, the height of plants, the length of roots, and the dry matter produced were not so great as in the cultures containing salts in low concentrations.

TABLE XI.—*Effect of concentration of salts in solution cultures on the growth of wheat seedlings. Average of 45 glasses for each concentration, with sodium sulphate, sodium carbonate, sodium chlorid, calcium chlorid, potassium chlorid, potassium nitrate, magnesium nitrate, and sodium nitrate in various combinations*

Concentration of salts in solution.	Number of plants alive.	Number of leaves per plant.	Height of plants.	Length of roots.	Ratio of height to length of root.	Dry weight of tops.	Dry weight of roots.	Ratio of weight of tops to roots.
P. p. m.			*Inches.*	*Inches.*		*Gm.*	*Gm.*	
None	9.7	1.97	7.5	3.9	1.92:1	0.123	0.052	2.36:1
1,000	9.0	1.91	8.6	4.4	1.91:1	.143	.046	3.37:1
2,000	7.8	1.72	6.8	3.4	1.96:1	.123	.044	3.04:1
3,000	5.1	1.67	6.8	3.6	1.88:1	.137	.048	3.08:1
4,000	5.7	1.41	6.0	3.2	1.87:1	.123	.045	2.83:1
5,000	5.7	1.70	5.4	3.0	1.88:1	.118	.052	2.40:1
6,000	5.8	1.62	5.7	3.1	1.90:1	.133	.050	2.67:1
7,000	4.1	1.34	4.6	2.8	1.81:1	.100	.040	2.43:1
8,000	4.3	1.43	4.1	2.3	1.74:1	.096	.038	2.46:1
9,000	4.4	1.37	4.1	2.3	1.74:1	.105	.040	2.58:1
10,000	3.8	1.29	3.2	2.0	1.70:1	.100	.043	2.37:1

Table XII shows the effect of the individual salts when used alone. The results given in this table are the averages of various concentrations, from 1,000 to 10,000 p. p. m. In interpreting these figures it must be remembered that no nutrient solution was added where the single salt was present. Using the average height of plants as an index, the toxicity of the salts was in the following order: Sodium carbonate, sodium chlorid, magnesium nitrate, sodium sulphate, magnesium chlorid, sodium nitrate, potassium nitrate, potassium chlorid, and calcium chlorid.

TABLE XII.—*Growth of wheat seedlings in solution cultures of various salts. Average of 10 concentrations of each salt*

Salt.	Number of plants alive.	Average leaves per plant.	Height of plants.	Length of roots.	Ratio of height to root length.	Dry weight of tops.	Dry weight of roots.	Ratio of weight of tops to roots.
			In.	*In.*		*Gm.*	*Gm.*	
Sodium sulphate	4.8	1.4	4.2	2.2	1.91:1	0.096	0.044	2.18:1
Sodium carbonate	1.7	1.2	2.1	1.6	1.31:1	.063	.028	2.25:1
Sodium chlorid	5.2	1.3	3.1	2.0	1.55:1	.092	.046	2.00:1
Calcium chlorid	8.4	1.8	7.9	3.2	1.88:1	.130	.066	1.97:1
Magnesium chlorid	6.0	1.6	5.0	1.5	3.33:1	.109	.036	3.03:1
Potassium chlorid	7.1	1.6	6.2	2.6	2.38:1	.126	.051	2.47:1
Potassium nitrate	6.0	1.8	5.8	2.7	2.15:1	.154	.039	3.95:1
Magnesium nitrate	2.5	1.3	3.4	1.5	2.27:1	.073	.031	2.35:1
Sodium nitrate	4.4	1.5	5.4	2.7	2.00:1	.113	.041	2.76:1

A rather conspicuous point in the table is the high ratio of tops to roots, both as to length and weight, in the cultures containing magnesium chlorid. The roots were also very short with magnesium nitrate,

even more so than with sodium carbonate. This affirms the well-known toxicity of magnesium salts to roots when used alone. The various salts in solution cultures did not act at all in the same manner as in soils, which shows the inadvisability of applying too widely to the soil the results obtained with solution cultures of alkali.

RESULTS OF STUDIES

NUMBER OF SEEDS GERMINATED

In the five graphs which follow (fig. 26–30) the effects of various factors on the number of seeds germinating in each glass are given. These are all summaries and each one represents a great many figures. It will be remembered that 10 seeds were planted in each glass.

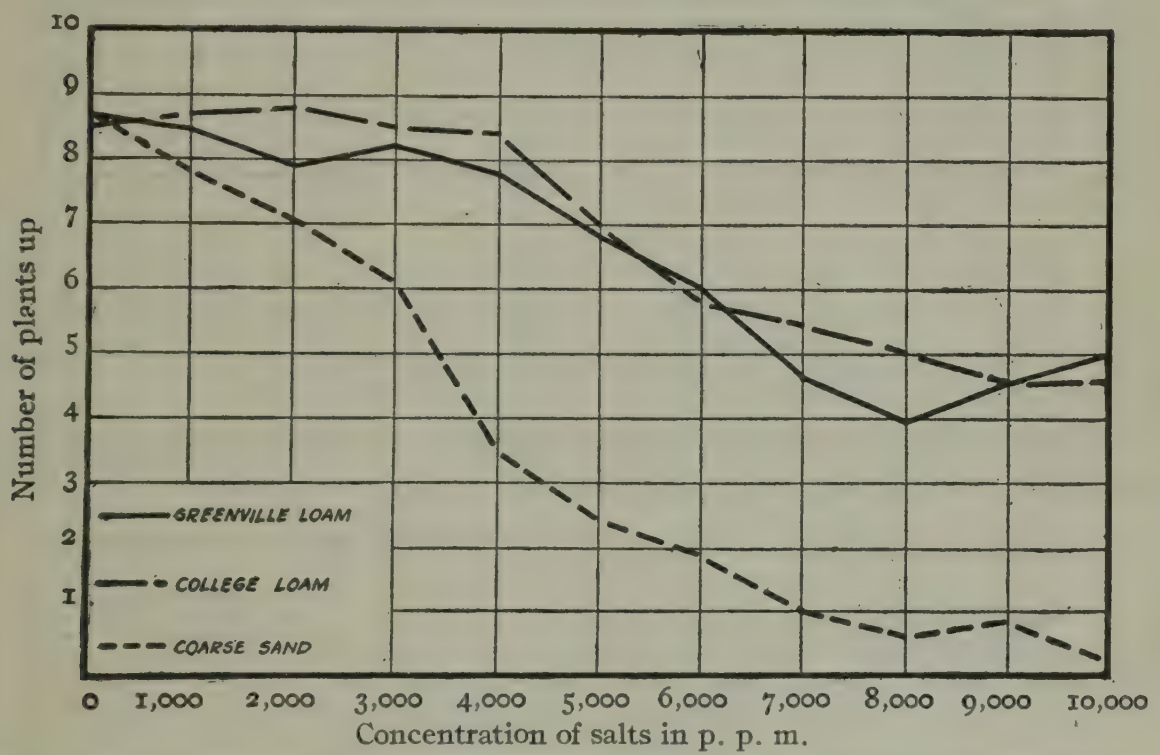

FIG. 26.—Curve showing the number of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

Figure 26 shows the effect of the concentration of salts in sand, Greenville loam, and College loam on the number of seeds germinating. Each curve represents the average of 13 salts in various combinations. In all of the soils there was an average of about 8½ plants coming up in each glass to which no salt was added. In sand the germination rapidly decreased with the concentration of salt, especially above 3,000 p. p. m. In College loam and Greenville loam there was but little falling off in germination until a concentration of over 4,000 p. p. m. had been reached.

Figure 27 shows the effect of the various salts on the germination of wheat in the three kinds of soil. Each salt represents the average of 10 concentrations ranging from 1,000 to 10,000 p. p. m. In sand there

was no germination whatever when ammonium carbonate was present even in as low a concentration as 1,000 p. p. m., but in the loams this

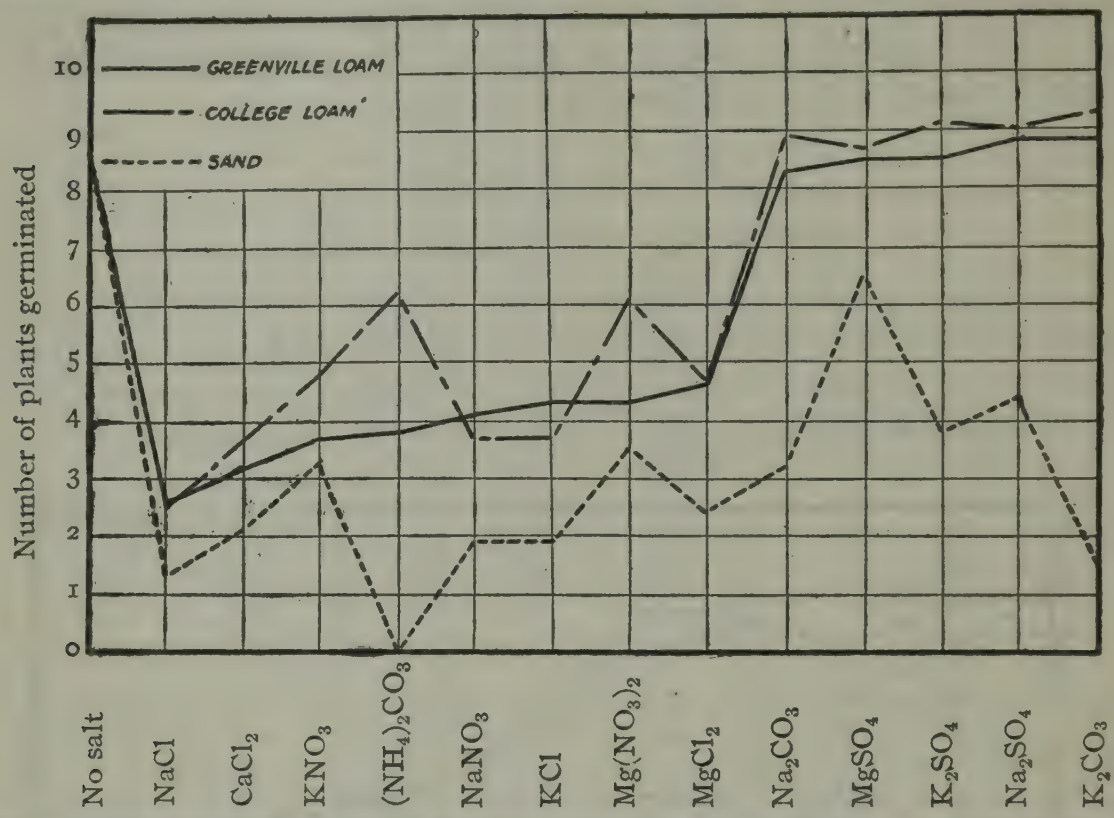

FIG. 27.—Curve showing the number of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

salt was not so toxic as some of the chlorids. The salts are arranged in the order of their toxicity to germination in Greenville loam.

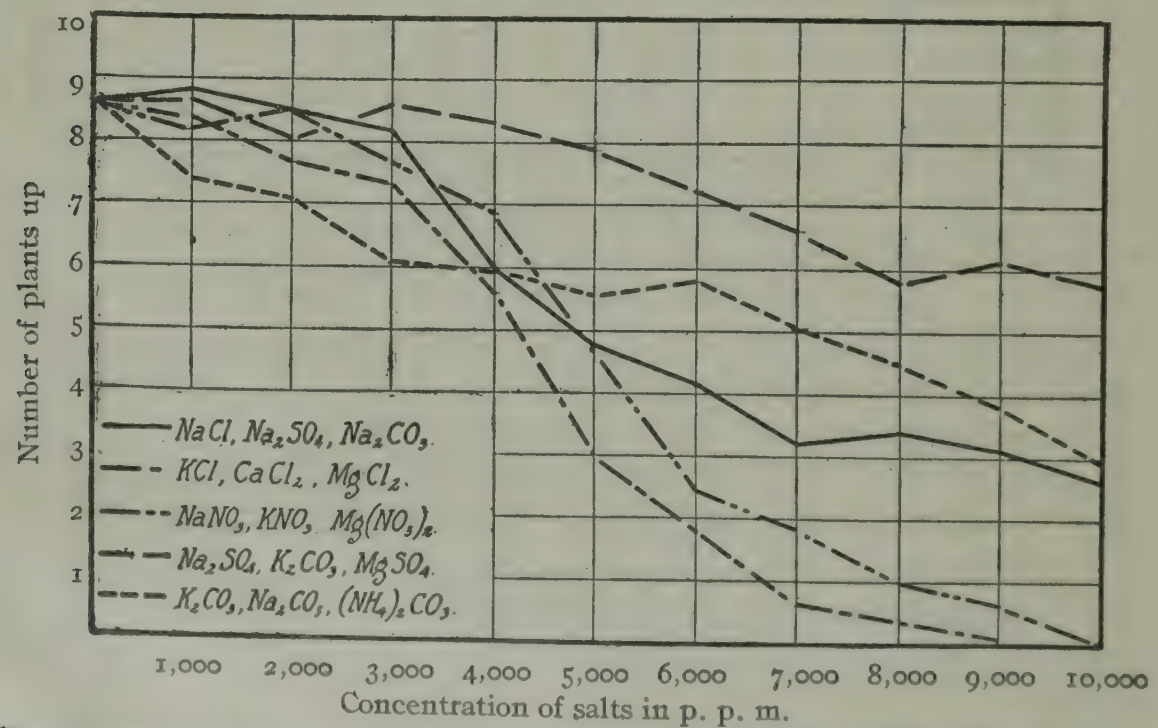

FIG. 28.—Curve showing the effect of various combinations of salts in different concentrations on the number of wheat plants germinating. Average of 15 combinations.

Figure 28 gives results where three salts were present in the soils in various combinations. Potassium chlorid, calcium chlorid, and mag-

nesium chlorid retarded germination most of any of the salts that were used together, while sodium sulphate, potassium sulphate, and magnesium sulphate retarded it least. With the first three salts there was no germination whatever above 9,000 p. p. m. and less than one-third complete germination at a concentration of 5,000 p. p. m.

In figure 29 the effect of the concentration of sodium chlorid, sodium carbonate, and sodium sulphate on the different crops is shown. A striking feature of the table is the stimulating effect of these salts in low concentration on the germination of sugar beets. With the exception of sugar beets, all the crops showed considerable similarity. One reason for the high germination of beets is the number of germs in each seed

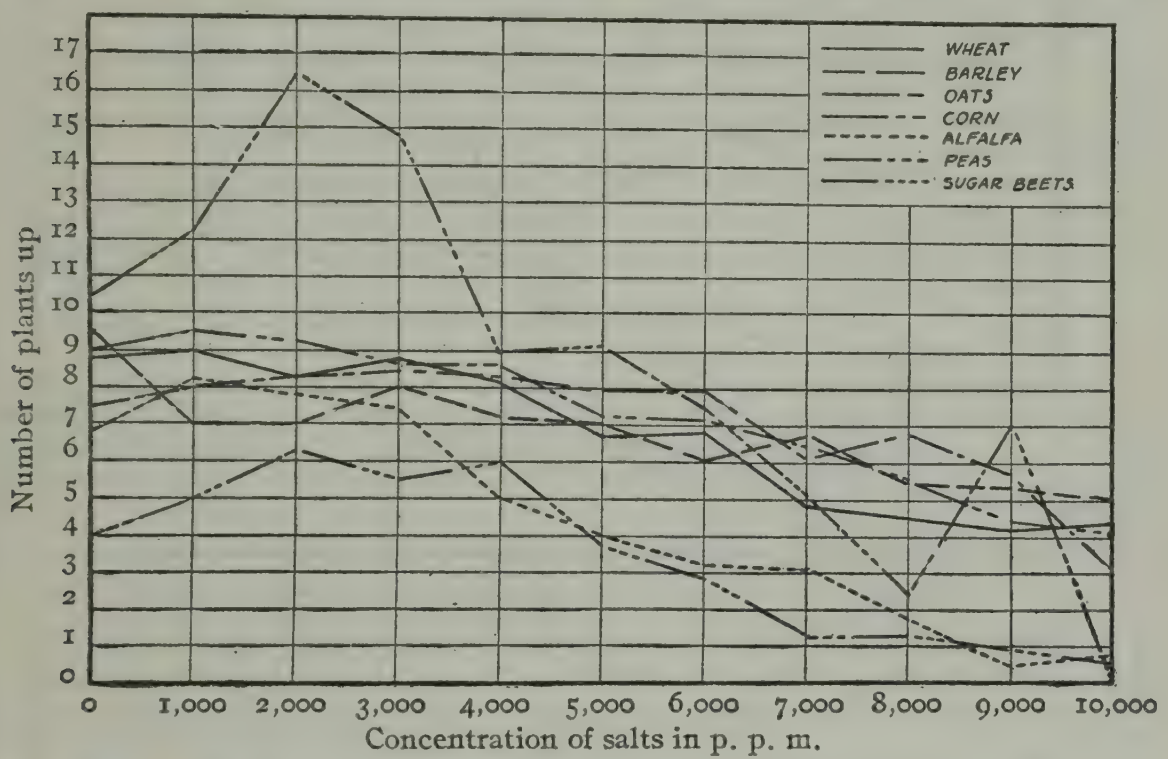

FIG. 29.—Curve showing the effect of concentration of salts on the number of seeds of various kinds germinating. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

ball. Alfalfa and field peas were affected by the salts decidedly more than the cereals.

The individual effect of sodium chlorid, sodium carbonate, and sodium sulphate on the different crops is shown in figure 30. Sodium chlorid is seen to be rather uniformly toxic to all crops, while sodium carbonate varies greatly. Sugar beets seem to be particularly resistant to sodium sulphate.

DRY MATTER PRODUCED

The five curves which follow (fig. 31–35) show the same results for amounts of dry matter produced by each glass that were given for germination in the five preceding figures (fig. 25–30). The numbers given represent the dry weight of plant material produced in each glass.

Figure 31 shows that the production of dry matter was stimulated by the presence of 1,000 p. p. m. of salt in the Greenville and College loam, but was about the same in sand for 1,000 p. p. m. as where no salt was

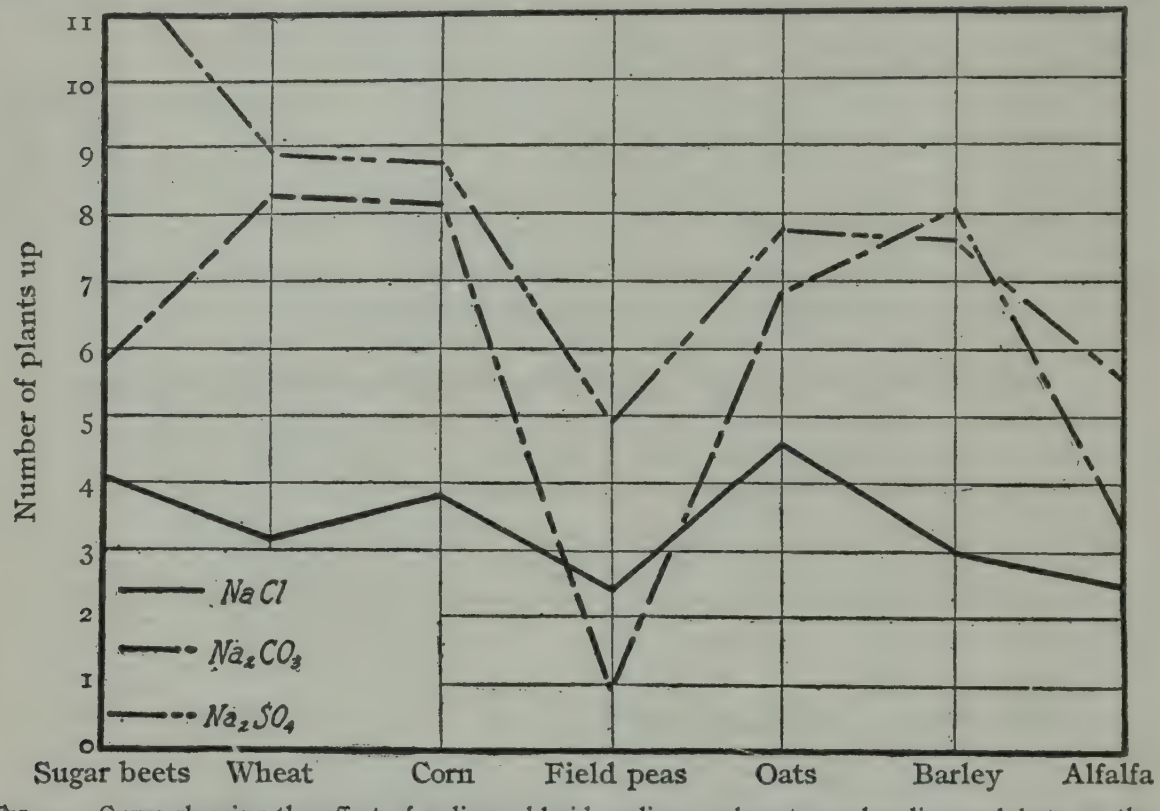

FIG. 30.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the number of plants up from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

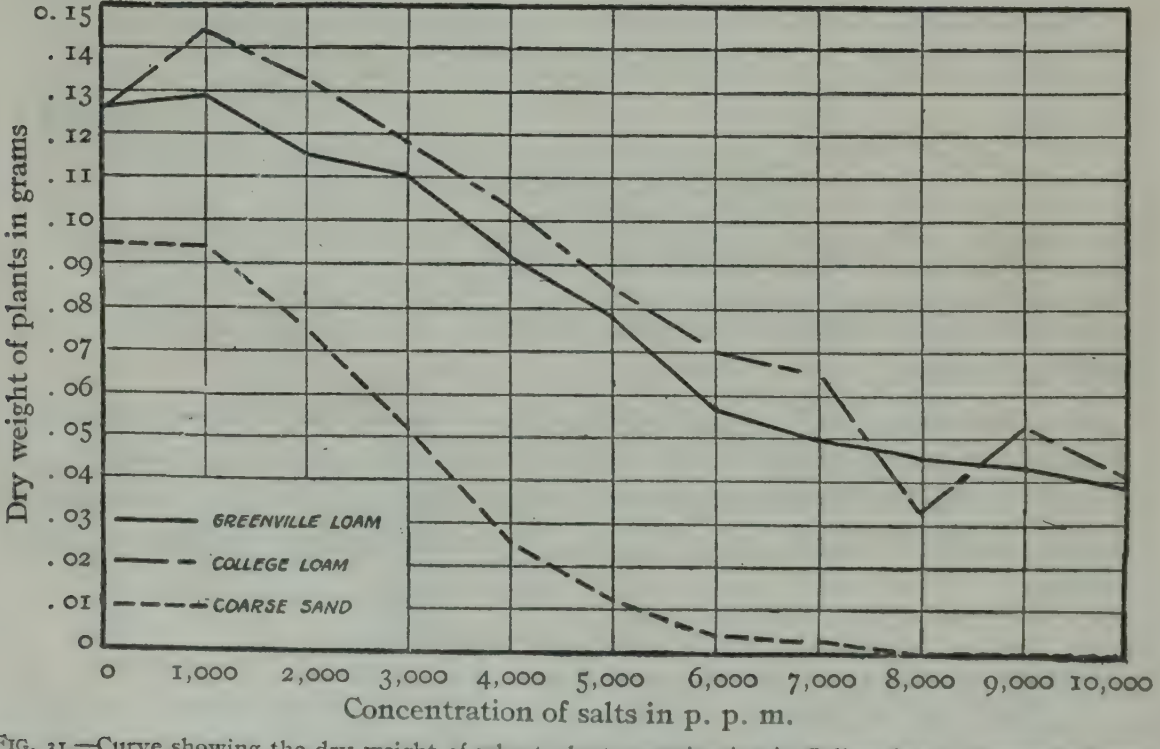

FIG. 31.—Curve showing the dry weight of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

added. The quantity of dry matter rapidly decreased with the concentration of salt above this point. In sand there was no plant growth at all above 8,000 p. p. m. of salt.

The effect of individual salts is shown in figure 32. A comparison of this graph with figure 27 shows that the dry matter is affected by the

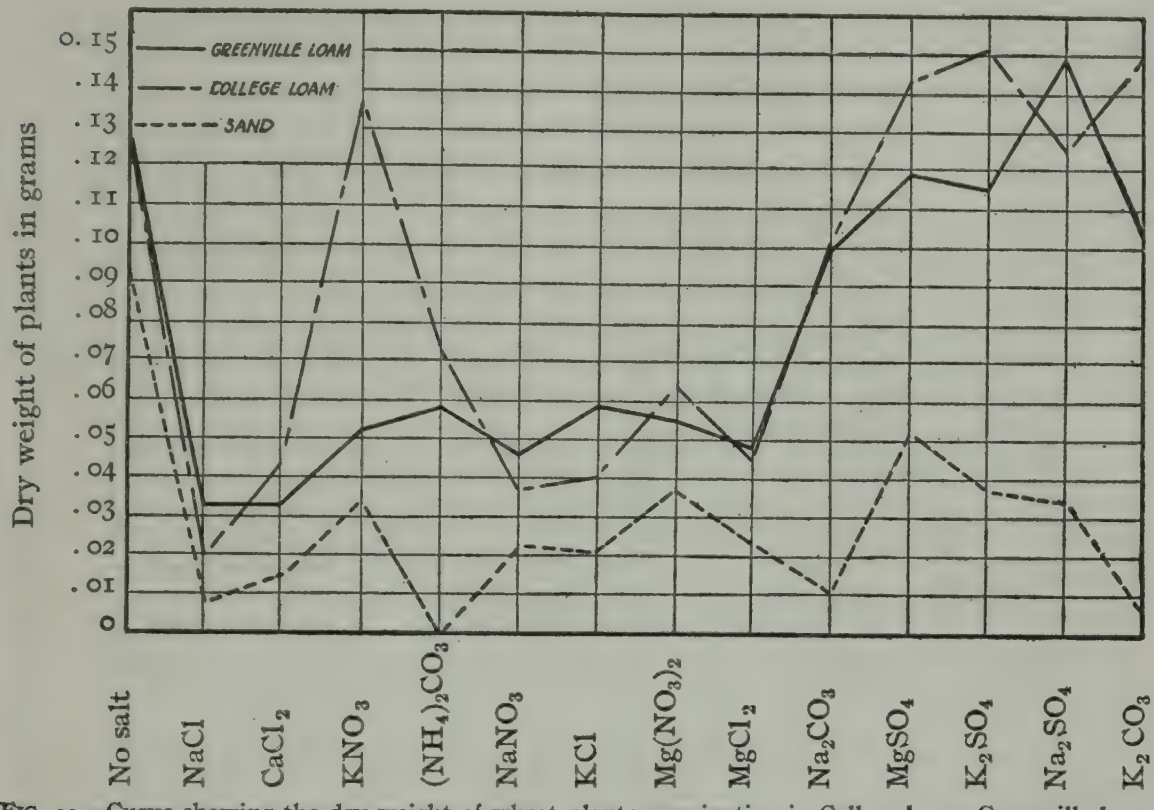

FIG. 32.—Curve showing the dry weight of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

salt in just about the same way as the germination. The greater relative toxicity of the carbonates in sand than in loam is again brought out.

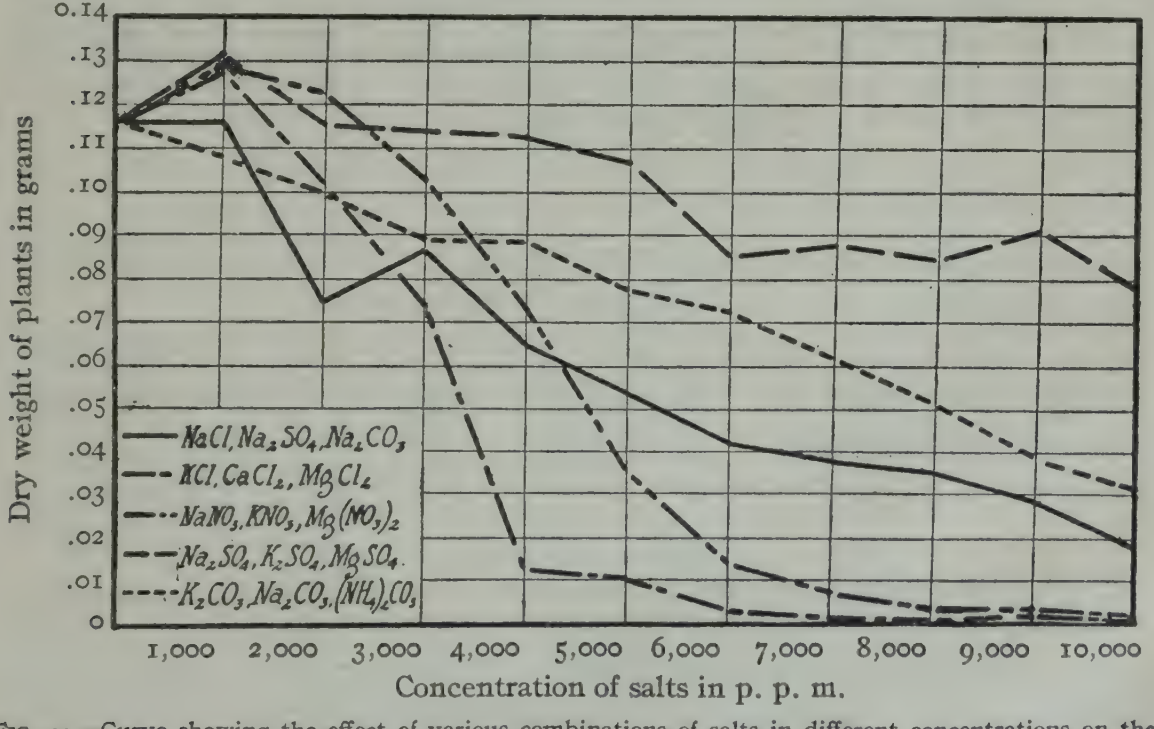

FIG. 33.—Curve showing the effect of various combinations of salts in different concentrations on the amount of dry weight produced. Average of 15 combinations of each 3 salts.

The action of each three salts used together is shown in figure 33. With the exception of potassium carbonate, sodium carbonate, and am-

monium carbonate the production of dry matter was stimulated by low concentrations of the salts. The growth of plants was not greatly reduced by the sulphates even in relatively high concentrations, while with the chlorids the yield dropped very rapidly and was practically nothing where the concentration was above 4,000 p. p. m.

Figure 34 shows the dry matter produced by different kinds of crops in soils containing sodium chlorid, sodium carbonate, and sodium sulphate in concentrations from 1,000 to 10,000 p. p. m. Corn gave by far the largest quantity of dry matter, but it was probably as much affected by the salt as any other crop. The yield was reduced from above 0.6 gm. per glass with no salt to less than 0.1 gm. per glass with a concentration of 10,000 p. p. m. Canada field peas produced a large quantity of dry

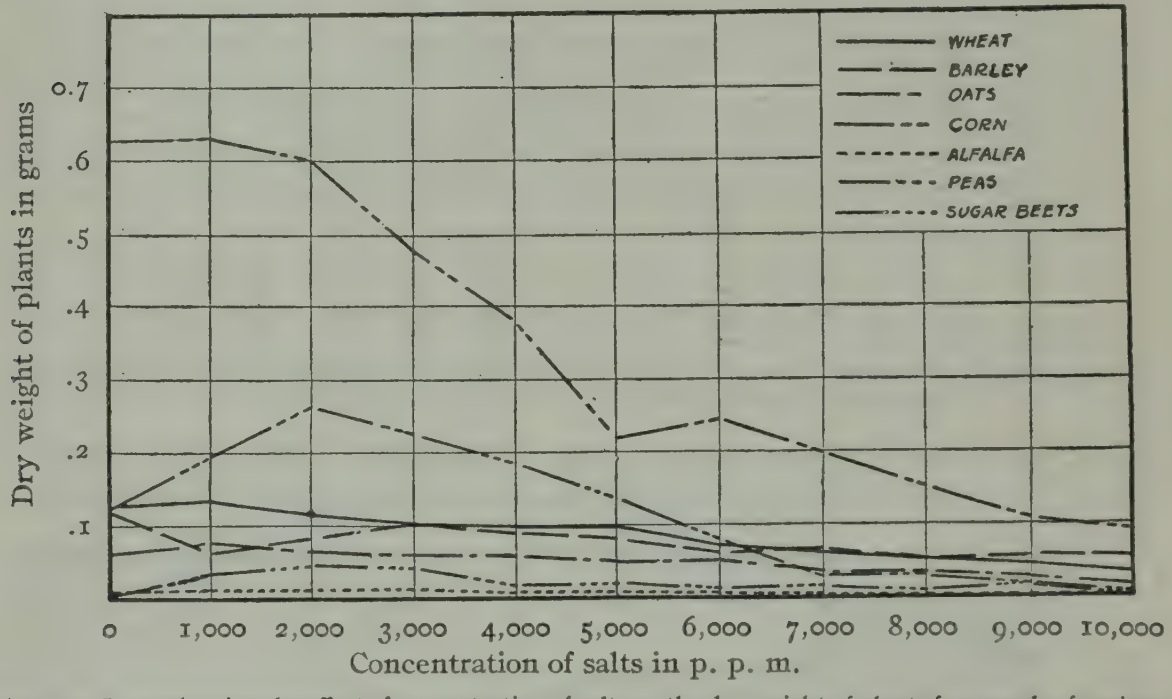

FIG. 34.—Curve showing the effect of concentration of salts on the dry weight of plants from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

matter, but they were also greatly affected by the concentration of salt. Alfalfa gave the least total yield under all conditions.

The effect of the individual salts on the yield of the various crops is brought out in figure 35. The yield of all crops was highest with sodium sulphate and lowest with sodium chlorid. With most crops it was only about half as great with sodium chlorid as with sodium carbonate.

DAYS TO COME UP

During the experiments a count was made each day of the number of plants that appeared above the surface of the soil, and from these figures a determination was made of the average time required for the plants in each glass to come up. The average results are in some cases misleading, because with toxic salts no plants germinated in the high concentration, and the averages were determined from the plants that came

up, which in this case were only those in low concentrations. At the same time there might be considerable germination in the high concentrations of less toxic salts, but the time of germination was increased. Thus, the average time of germination might appear to be longer in the less toxic salt, when in reality this would not be the case.

Figure 36 shows the time required for wheat to come up in Greenville loam, College loam, and sand containing salts in concentrations up to 10,000 p. p. m. The results are the average for 13 different salts. The time required to germinate where no salt was present varied from about 5½ to 6½ days with no salt and from 10½ to 15 days with 10,000 p. p. m.

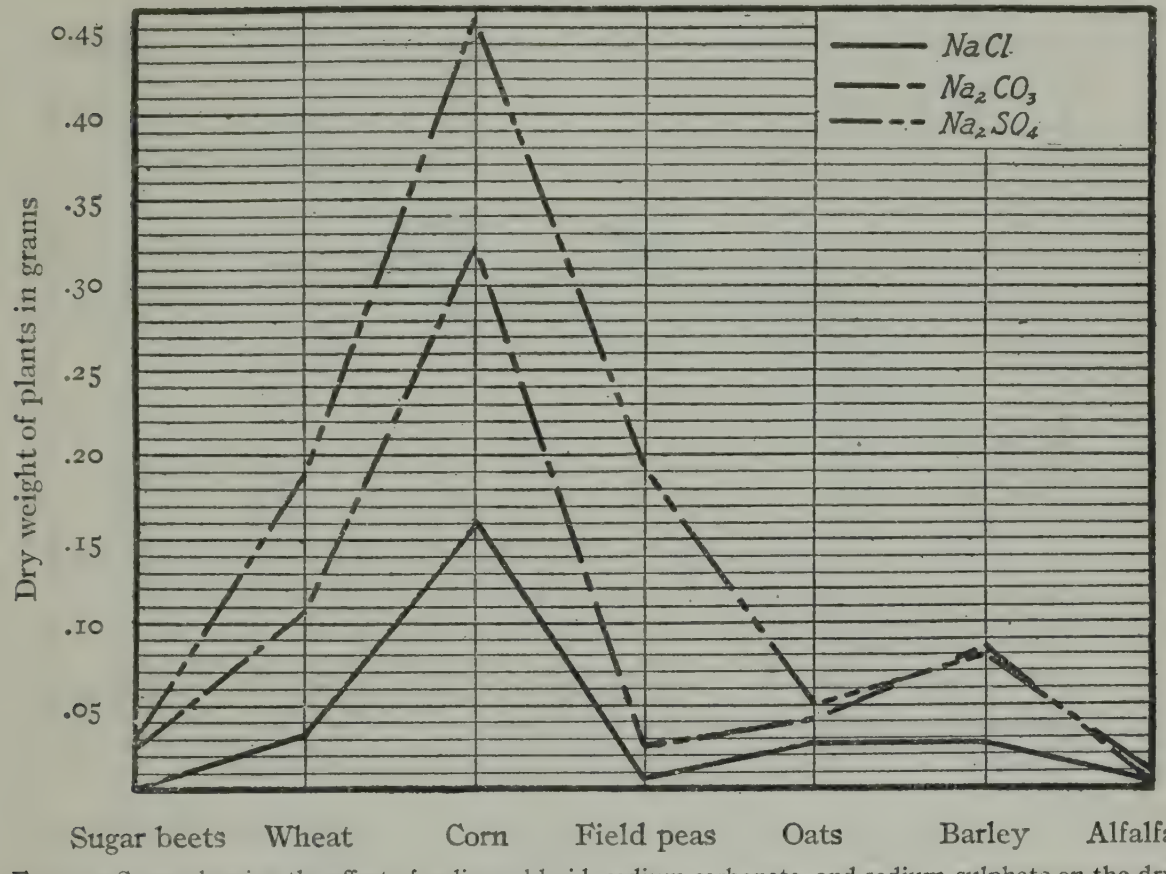

FIG. 35.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the dry weight from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

of salt. The time was doubled by the presence of from 6,000 to 8,000 p. p. m. of salt.

Figure 37 shows the effect of individual salts on time of germination in the three kinds of soil. Calcium chlorid, magnesium chlorid, and sodium chlorid retarded germination most in Greenville soil, while sodium nitrate came next.

In sand the salts did not retard germination as much as in loam. This is because there was no germination whatever in sand with the highest concentration. There was no germination in sand when ammonium carbonate was added, even in as low concentrations as 1,000 p. p. m.

The results where three salts were used together are shown in figure 38. The average time of germination with potassium chlorid, calcium

chlorid, and magnesium chlorid in a concentration of 8,000 p. p. m. was over 20 days, which was nearly four times as long as the time required for seeds to come up where no salt was added. The period of germination was less with the sulphates and carbonates than with the other salts.

The time of germination of different crops in the presence of sodium chlorid, sodium carbonate, and sodium sulphate in combination is shown in figure 39. Where no salts were added, the time varied from about 4½ days for barley to nearly 8 days for sugar beets. The same general relation between the germination of various crops continued with the different concentrations of salts. Alfalfa was least affected by salts of any of the crops in the length of its germination period.

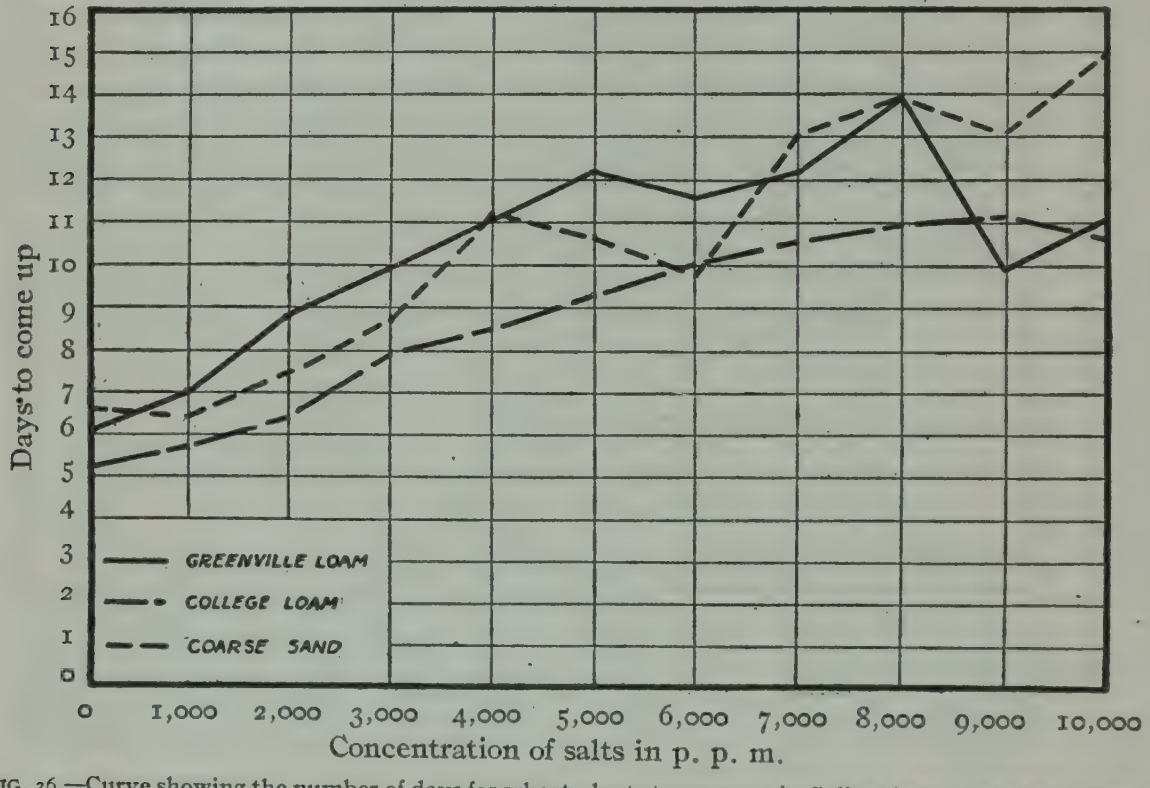

FIG. 36.—Curve showing the number of days for wheat plants to come up in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

Figure 40 shows the effects of individual salts on the germination period of different crops. This brings out again the fact already mentioned, that the same relative toxicity of salts does not hold for all crops.

HEIGHT OF PLANT

Figures 41, 42, 43, 44, and 45 show the effect of various factors on the height of plants. This is probably one of the best means of comparison for young plants of this kind.

Plants growing in sand were not so high in any case as those growing in other soils; in the Greenville loam they were slightly higher than in College loam. The height in loam was greater with 1,000 p. p. m. of salt than where no salt was added, but above this point the height decreased considerably as the concentration of salt increased. In sand

the height was much more affected by the salts than in loam. The rise in the curve at 10,000 p. p. m. is due to the fact that no plants grew at this concentration in the more toxic salts and not to the actual increase in height.

Figure 42 shows the effect of each salt in the three soils on the height of wheat. The same general results which have already been pointed out in connection with germination and dry-matter production are noted here. Potassium nitrate produced the shortest plants in the loams,

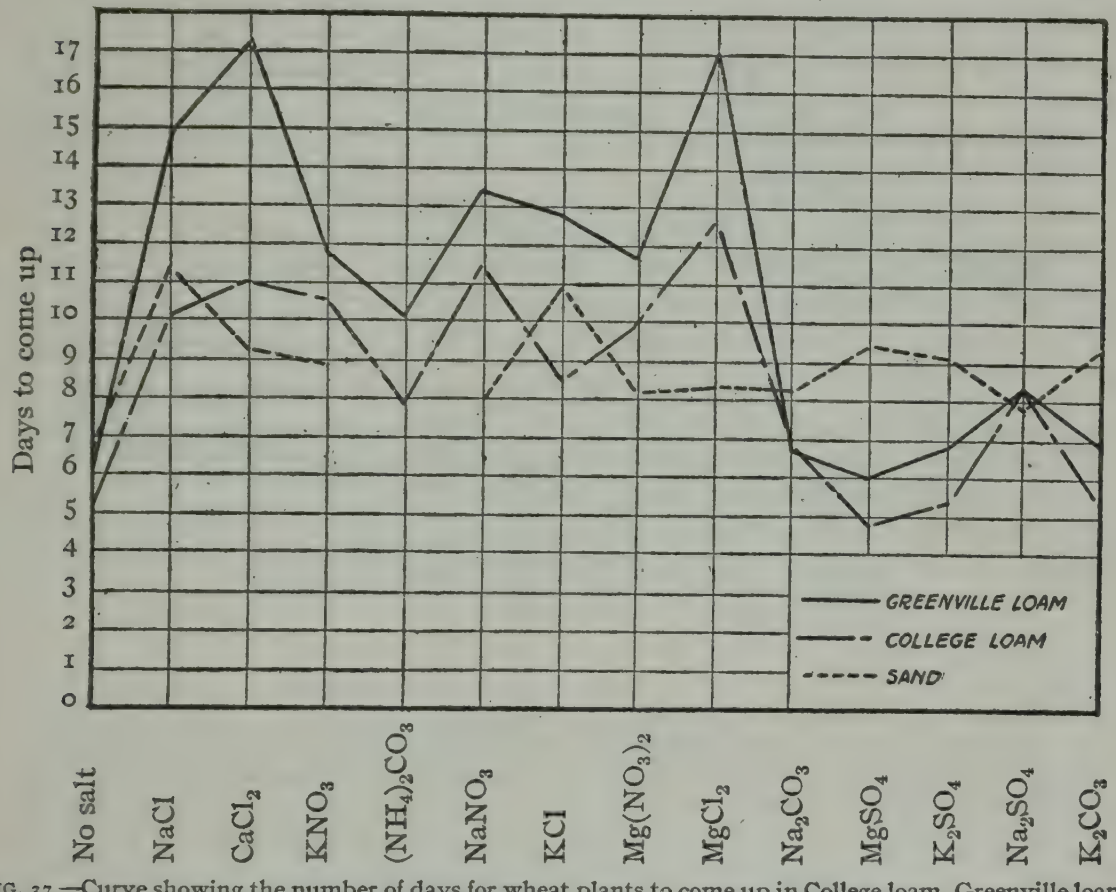

FIG. 37.—Curve showing the number of days for wheat plants to come up in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

while sodium chlorid and sodium carbonate produced the shortest plants in sand.

Figure 43 shows the height of plants in soils to which three salts in combinations of various kinds had been added. This diagram shows that the chlorids and nitrates had a great effect on the height of plants, while the carbonates and sulphates had less.

The effect of the concentrations of sodium chlorid and sodium sulphate on the height of different crops is shown in figure 44. While the curves are somewhat irregular, they show the same results that have already been brought out regarding the shortening of plants by alkali.

Figure 45 shows the effect of individual salts on the height of various crops. It will be noted that in practically all cases the crops were shorter where sodium chlorid was present than with the other salts; also that sodium sulphate usually gave the highest plants.

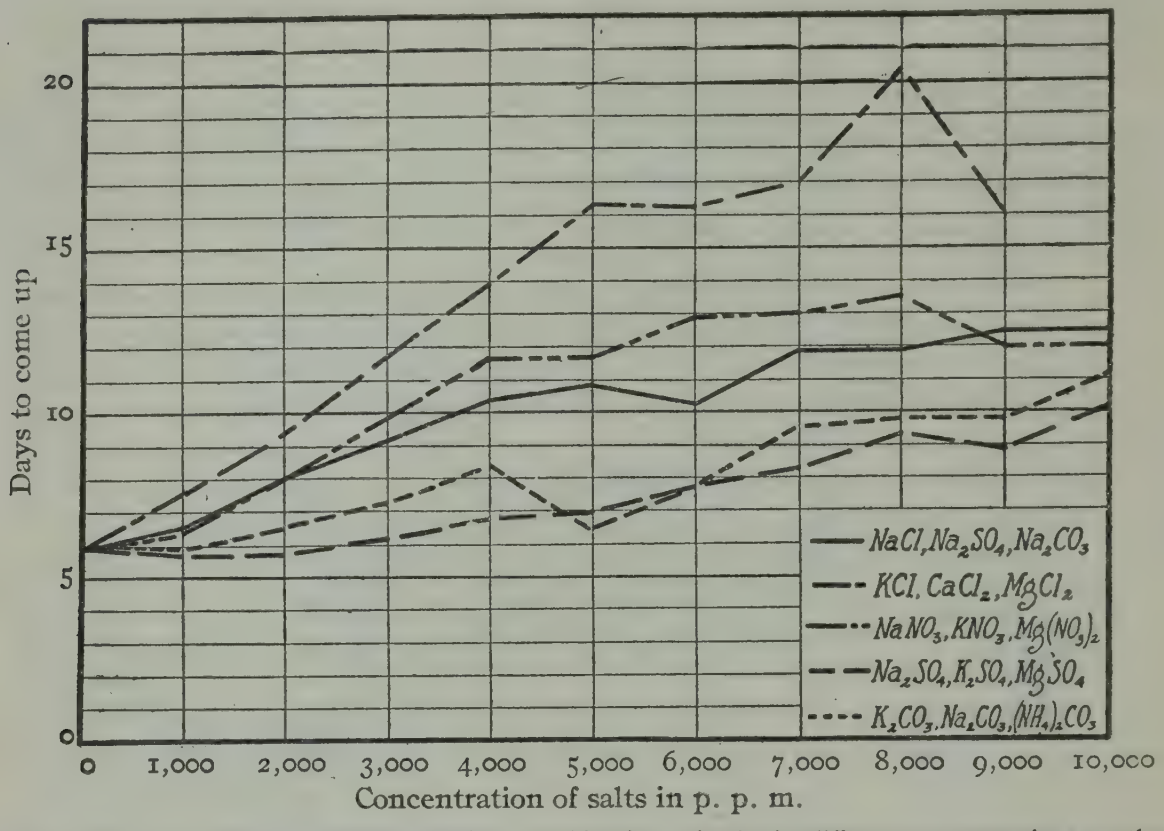

FIG. 38.—Curve showing the effect of various combinations of salts in different concentrations on the number of days to come up. Average of 15 combinations.

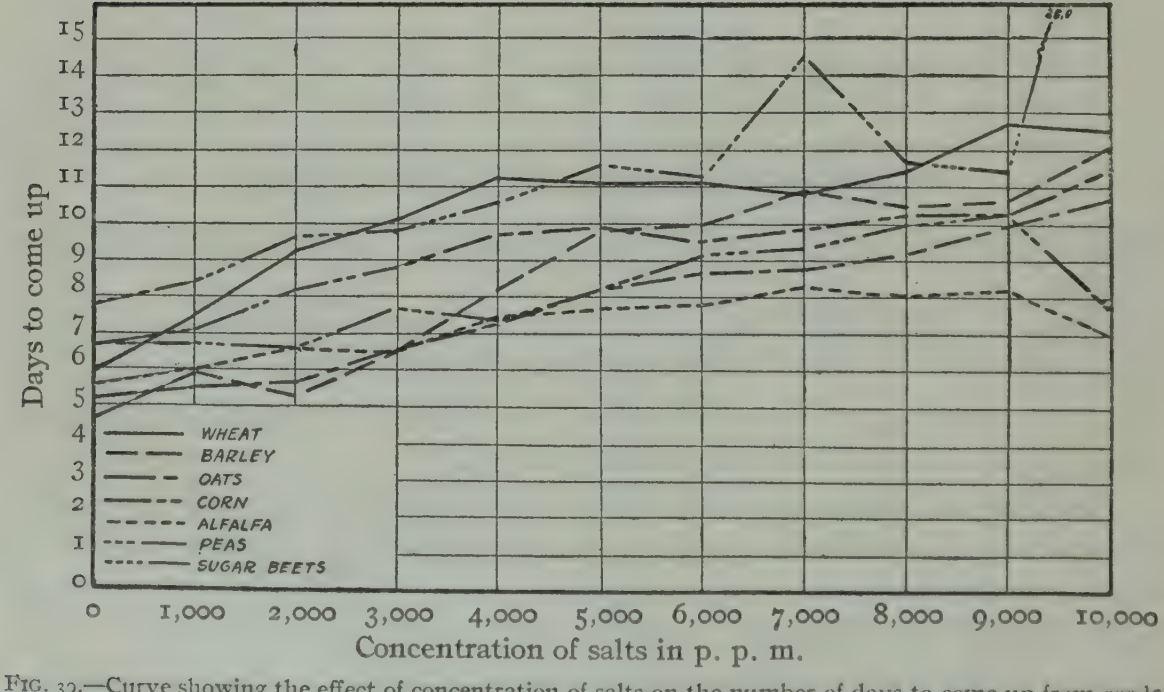

FIG. 39.—Curve showing the effect of concentration of salts on the number of days to come up from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

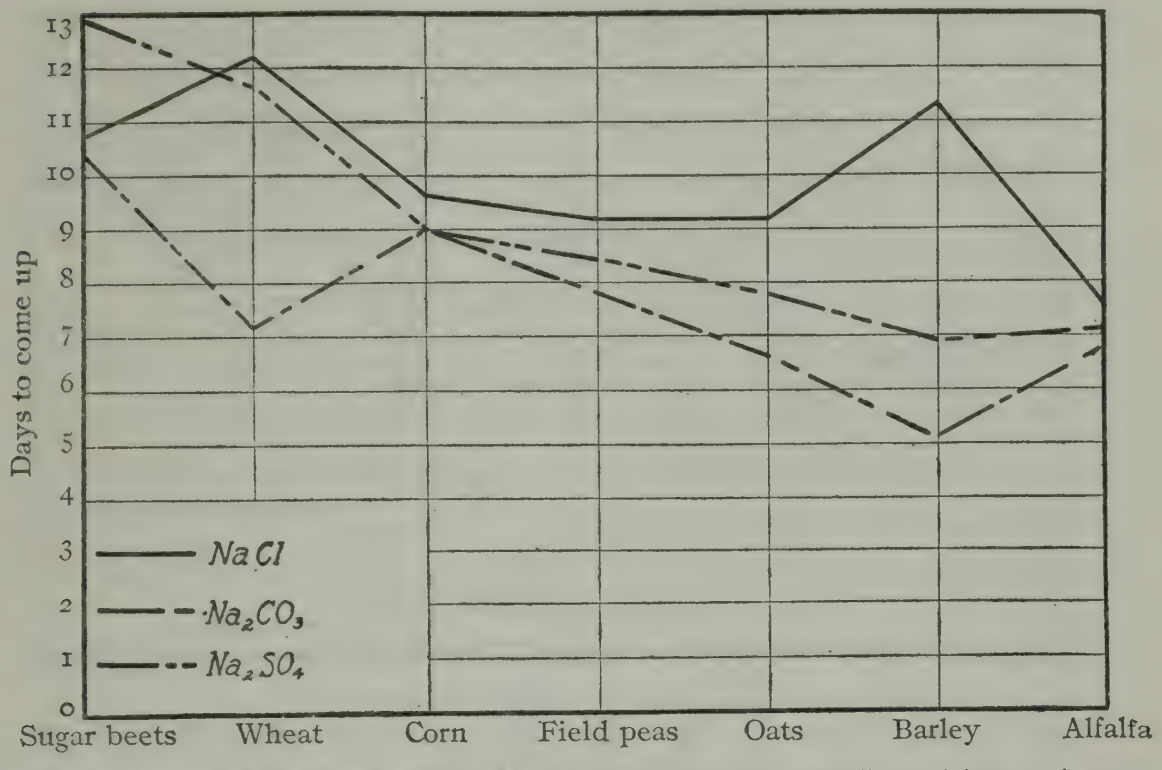

FIG. 40.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the number of days to come up from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

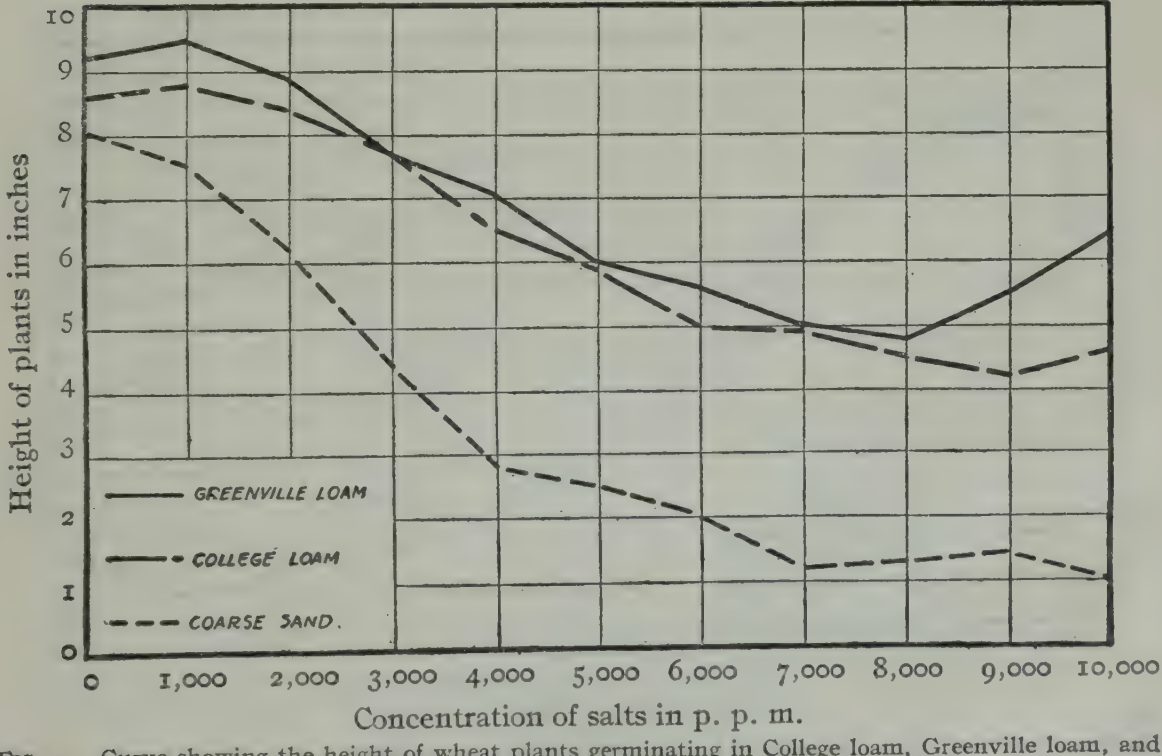

FIG. 41.—Curve showing the height of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

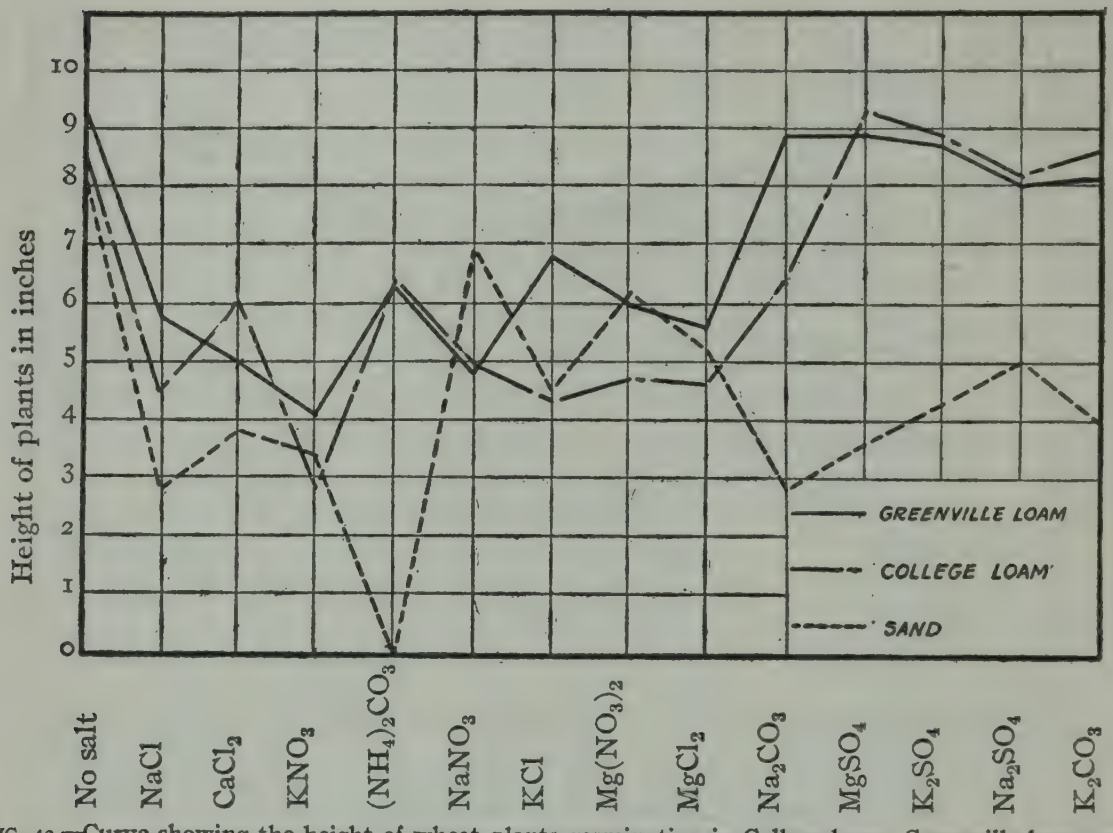

FIG. 42.—Curve showing the height of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

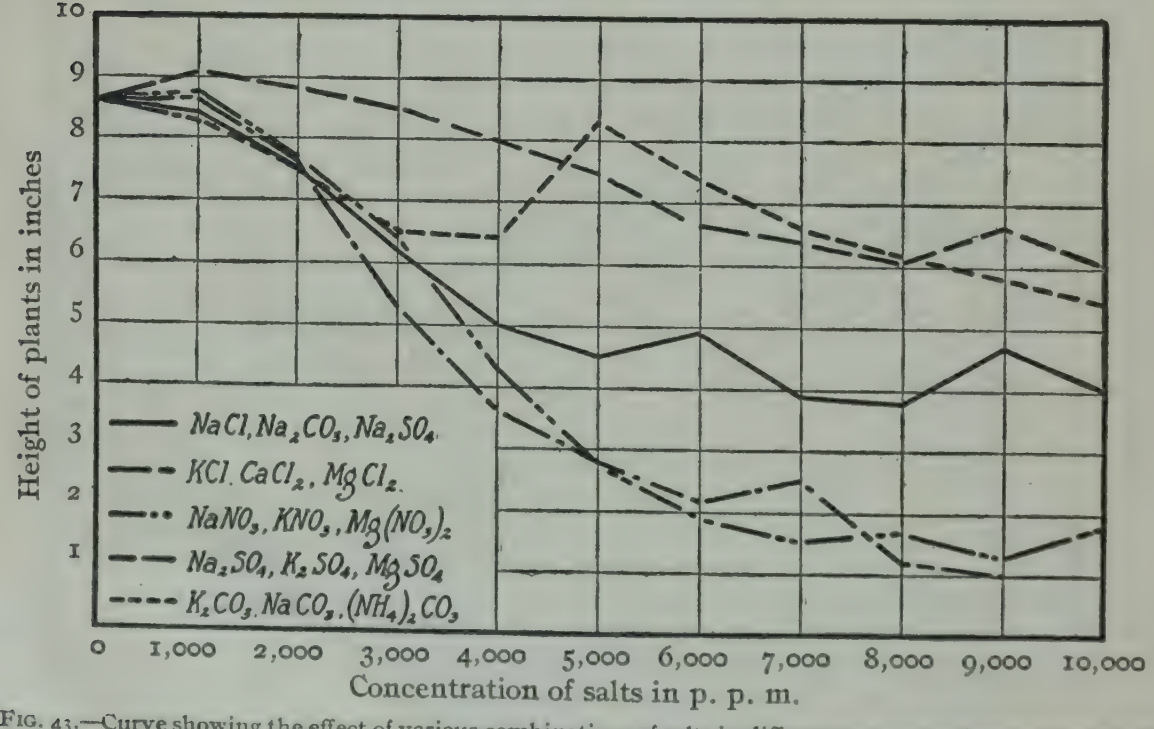

FIG. 43.—Curve showing the effect of various combinations of salts in different concentrations on the height of plants. Average of 15 combinations of each group of 3 salts.

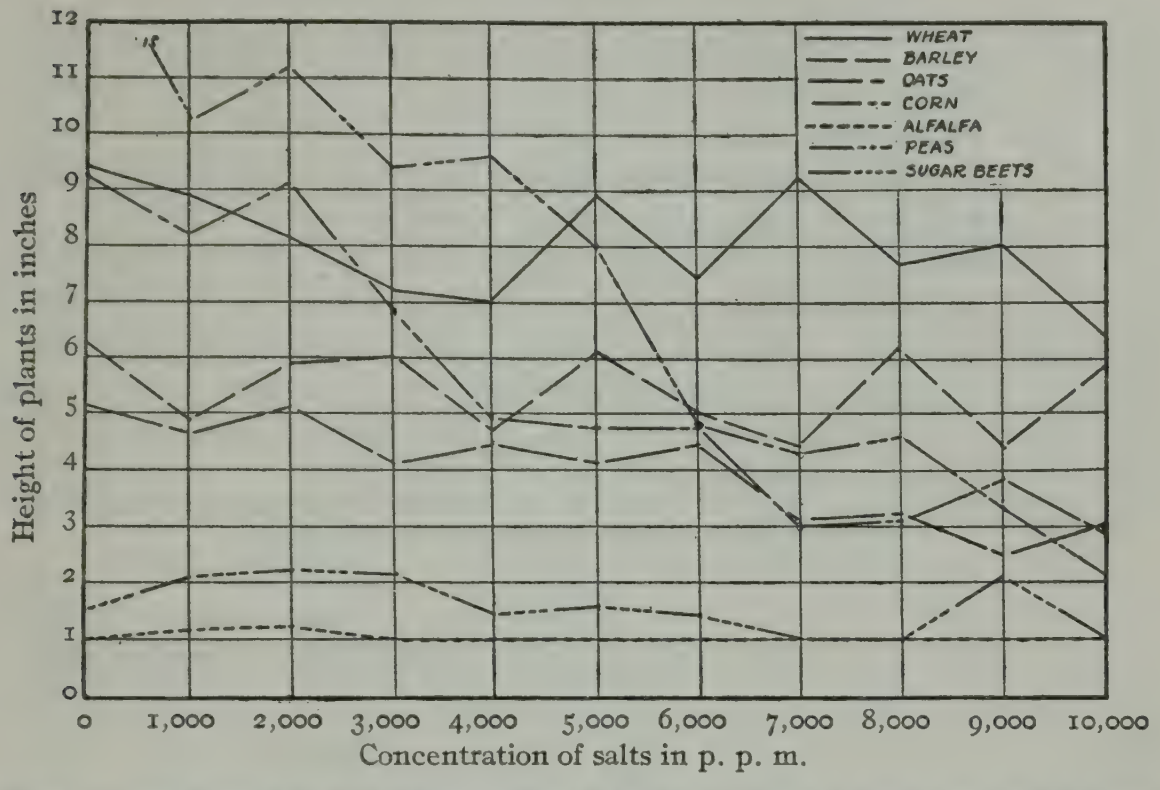

FIG. 44.—Curve showing the effect of concentration of salts on the height of plants from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

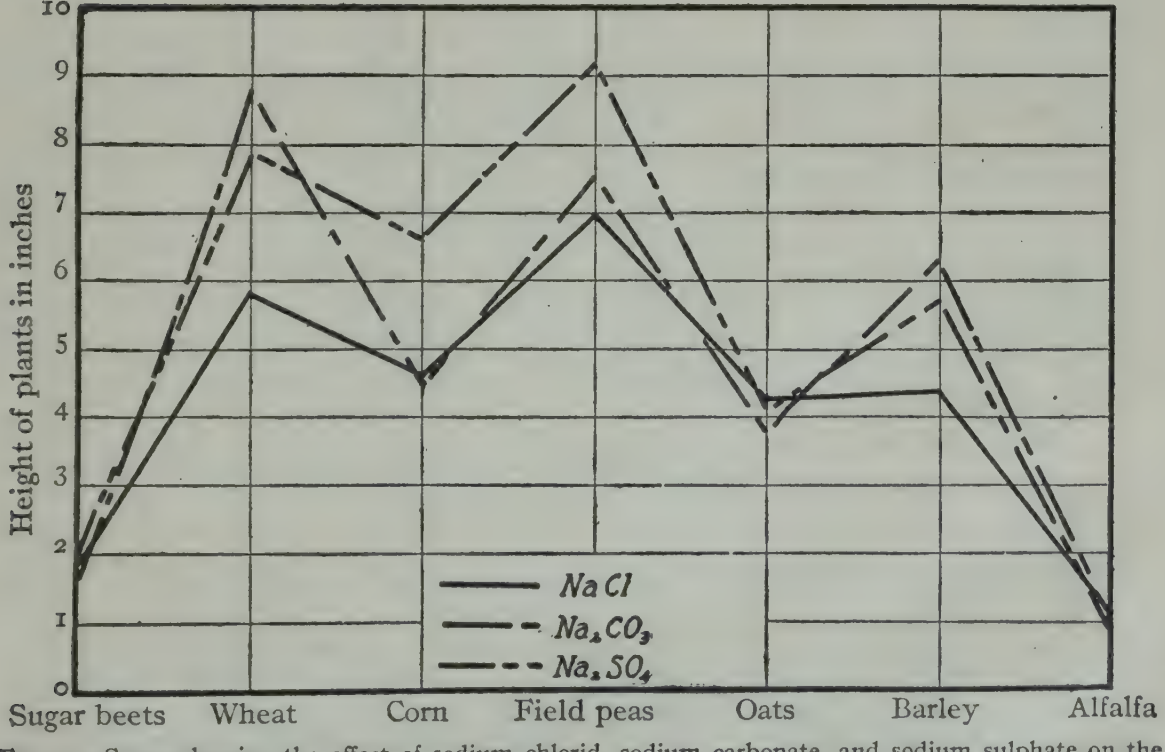

FIG. 45.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the height of plants from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

ACTION OF THE VARIOUS IONS

COMPARISONS OF CATIONS AND ANIONS

In order to determine the effect of the different ions and to compare the relative action of the cations and anions, the results of the various tests were summarized and are presented in Tables XIII and XIV. These data represent the averages of the various concentrations of the salts in three different soils; hence, they should be fairly reliable.

On examining Table XIII it will be seen that the chlorid was by far the most toxic anion, followed by the nitrate, carbonate, and sulphate in the order named. This order held for all salts regardless of the basic ion, and is contrary to ideas on the subject previously held, as the carbonate was thought by many writers to be most injurious.

TABLE XIII.—*Effect of various anions on the germination and growth of wheat. Average for 3 soils and 10 concentrations for each soil*

Ions.	Number of trials.	Number of plants germinated.	Days to come up.	Average height of plants.	Average number of leaves per plant.	Weight of dry matter per glass.
				Inches.		*Gm.*
Sodium—						
Chlorid	30	2.3	11.2	4.3	1.35	0.020
Sulphate	30	7.0	9.0	7.0	1.77	.101
Carbonate	30	6.2	7.7	5.9	1.67	.072
Nitrate	30	3.3	8.6	5.5	1.62	.035
Average of sodium salts.	120	4.7	9.1	5.7	1.60	.057
Potassium—						
Chlorid	30	3.1	11.6	5.2	1.54	.040
Sulphate	30	7.1	6.5	7.3	1.75	.101
Carbonate	30	6.4	5.8	6.9	1.61	.087
Nitrate	30	3.7	9.0	3.4	1.29	.074
Average of potassium salts	120	5.1	8.5	5.7	1.55	.076
Magnesium—						
Chlorid	30	3.4	12.8	5.1	1.49	.039
Sulphate	30	7.9	6.7	7.3	1.72	.105
Carbonate	30	4.6	8.8	5.6	1.63	.052
Average of magnesium salts	90	5.3	9.4	6.0	1.61	.065
Calcium—						
Chlorid	30	2.8	12.1	4.9	1.66	.031
Ammonium—						
Carbonate	30	3.3	6.0	4.2	1.17	.044

In Table XIV a comparison is made of the various cations. Sodium is seen to be most injurious of all the bases except ammonium. Sodium is followed by calcium, potassium, and magnesium in the order named. This same order of toxicity held with all the acid radicals that were tried.

TABLE XIV.—*Effect of various cations on germination and growth of wheat. Average for 3 soils and 10 concentrations for each soil*

Cations.	Number of trials.	Number of plants germinated.	Days to come up.	Average height of plants.	Average number of leaves per plant.	Weight of dry matter per plant.
				Inches.		*Gm.*
Chlorid—						
Sodium	30	2.3	11.2	4.3	1.35	0.020
Potassium	30	3.1	11.6	5.2	1.54	.040
Calcium	30	2.8	12.1	4.9	1.66	.031
Magnesium	30	3.4	12.8	5.1	1.49	.039
Average of chlorids	120	2.9	11.9	4.9	1.51	.033
Sulphate—						
Sodium	30	7.0	9.0	7.0	1.77	.101
Potassium	30	7.1	6.5	7.3	1.75	.101
Magnesium	30	7.9	6.7	7.3	1.72	.105
Average of sulphates	90	7.3	7.4	7.2	1.75	.102
Carbonate—						
Sodium	30	6.2	7.7	6.0	1.67	.071
Potassium	30	6.4	6.8	6.9	1.61	.087
Ammonium	30	3.3	6.0	4.2	1.17	.044
Average of carbonates	90	5.3	6.8	5.7	1.48	.067
Nitrate—						
Sodium	30	3.3	8.6	5.5	1.62	.035
Potassium	30	3.9	9.0	3.4	1.29	.074
Magnesium	30	4.6	8.8	5.6	1.63	.052
Average of nitrates	90	3.9	8.8	4.8	1.51	.054

A comparison of the various data presented in Tables XIII and XIV brings out clearly the fact that the injurious effects of the alkali salts in soils may be attributed more to the anion, or acid radical, than to the cation, or basic radical. All the chlorids gave results very similar to each other. The same may be said of the sulphates and nitrates. The different salts of sodium or potassium, on the other hand, differed greatly, according to the acid radical combined with them. This is just opposite to the conclusions of Kearney and Cameron (13) based on solution cultures.

RELATION OF MOLECULAR WEIGHT IN TOXICITY

A number of workers have considered the toxicity of various alkali salts to be proportional to their osmotic pressure. In order to determine whether this were true, the different salts which had been tested were arranged in the order of their toxicity and the molecular weight of each placed opposite to ascertain whether there was any relation between the two. Of course, it is understood that the lower the molecular weight of a salt the more molecules there are in a solution containing a given per-

centage of salt, and the more molecules there are the greater will be the osmotic pressure, provided there is the same dissociation. Following out this reasoning, a salt of low molecular weight should be more toxic than one of higher molecular weight if the salts were present in the same percentage by weight. Indeed, in the study of osmosis, salts would not be expressed in percentages but in molecular solutions. In soils, however, it is impossible to express salts on a basis of molecular solution.

In Table XV it will be seen that in a general way salts with low molecular weights are more toxic than those having a higher molecular weight, but there are so many exceptions that this can not be considered a general law holding for all salts. For example, magnesium sulphate has a lower molecular weight than potassium sulphate, sodium sulphate, potassium carbonate, or magnesium nitrate, and yet it is less toxic than any of these salts.

TABLE XV.—*Comparison of the toxicity of the various salts with their molecular weight*

Salts in order of toxicity.	Number of plants germinated.	Weight of dry matter produced.	Molecular weight.
		Gm.	
Sodium chlorid	2.3	0.020	58.5
Calcium chlorid	2.8	.031	111.0
Potassium chlorid	3.1	.040	74.6
Sodium nitrate	3.3	.035	85.1
Ammonium carbonate	3.3	.044	202.2
Magnesium chlorid	3.4	.039	95.3
Potassium nitrate	3.9	.074	101.2
Magnesium nitrate	4.6	.052	148.4
Sodium carbonate	6.2	.071	106.1
Potassium carbonate	6.4	.087	138.3
Sodium sulphate	7.0	.101	142.2
Potassium sulphate	7.1	.101	174.4
Magnesium sulphate	7.9	.105	120.4

SALTS ALONE AND IN COMBINATION WITH OTHER SALTS

One of the most important questions arising in connection with the toxicity of alkali is regarding the action of salts when present alone and when in combination with other salts. Considerable work has been done on the antagonistic action of various salts in solution cultures, and some very remarkable results have been obtained; but many of these results do not hold when the salts are applied to the soil.

An examination of figures 2 to 24 will show that in the soil the antagonistic action of the various alkali salts is not so great as previous workers have found for these same salts in solutions. For example, the magnesium salts when used alone in solution are very toxic to plants, but this is largely overcome by the presence of other salts. The results for mag-

nesium salts in soils do not show them to be particularly toxic. This is probably due in part to the high lime content of the soils used.

An attempt is made in Table XVI to bring together a summary of results for salts applied to soil singly and in combination. These are grouped as sulphates, carbonates, nitrates, chlorids, and the sodium salts. Under each salt are given certain figures which, when multiplied by 1,000, give the parts per million of salt added to the soil. Each figure is the average for Greenville loam, College loam, and sand. The results include the number of plants germinating in each glass, the weight of dry plant material produced in each glass, the average height of plants, and the average number of days required for the plants to come up.

TABLE XVI.—*Effect of combination of salts on the germination and growth of wheat. Average of three soils*

[Figures under salts multiplied by 1,000 equal parts per million of salt in the soil]

Sulphates.								Carbonates.								Nitrates.								Chlorids.								Sodium salts.							
Sodium.	Potassium.	Magnesium.	Total salts.	Number of plants up.	Weight of dry matter produced.	Height.	Days to come up.	Sodium.	Potassium.	Ammonium.	Total salts.	Number of plants up.	Weight of dry matter produced.	Height.	Days to come up.	Sodium.	Potassium.	Magnesium.	Total salts.	Number of plants up.	Weight of dry matter produced.	Height.	Days to come up.	Calcium.	Potassium.	Magnesium.	Total salts.	Number of plants up.	Weight of dry matter produced.	Height.	Days to come up.	Sulphate.	Carbonate.	Chlorid.	Total salts.	Number of plants up.	Weight of dry matter produced.	Height.	Days to come up.
					Gm.	*In.*							*Gm.*	*In.*							*Gm.*	*In.*							*Gm.*	*In.*							*Gm.*	*In.*	
0	0	0	0	8.6	0.116	8.6	6.0	0	0	0	0	8.6	0.116	8.6	6.0	0	0	0	0	8.6	0.116	8.6	6.0	0	0	0	0	8.6	0.116	8.6	6.0	0	0	0	0	8.6	0.116	8.6	6.0
1	0	0	1	9.3	.127	8.1	6.7	1	0	0	1	8.3	.689	8.6	5.9	1	0	0	1	8.0	.118	8.3	8.1	1	0	0	1	8.3	.106	6.8	8.9	1	0	0	1	7.7	.092	5.9	7.2
0	1	0	1	9.7	.154	8.4	5.9	0	1	0	1	7.7	.095	6.9	6.5	0	1	0	1	8.7	.136	7.7	6.6	0	1	0	1	8.7	.140	9.2	6.7	0	1	0	1	9.0	.112	8.0	5.6
0	0	1	1	10.0	.135	9.3	5.0	0	0	1	1	5.3	.084	9.1	5.3	0	0	1	1	6.7	100	8.8	6.7	0	0	1	1	8.3	.111	7.5	8.6	0	0	1	1	7.7	.080	6.5	9.5
1	½	½	2	7.7	.090	8.7	5.6	1	½	½	2	8.3	.128	8.3	6.5	1	½	½	2	8.0	.110	8.1	6.9	1	½	½	2	7.0	.100	8.3	9.3	1	½	½	2	9.7	.123	6.4	6.0
½	1	½	2	8.0	.109	8.4	5.7	½	1	½	2	5.0	.095	11.1	5.3	½	1	½	2	8.7	.130	8.1	7.1	½	1	½	2	8.3	.121	8.0	9.3	½	1	½	2	9.3	.128	8.3	5.9
½	½	1	2	8.0	.118	9.5	6.2	½	½	1	2	6.3	.092	9.0	5.7	½	½	1	2	8.3	.126	8.4	7.4	½	½	1	2	6.0	.072	7.0	9.5	½	½	1	2	9.0	.114	8.1	7.5
1	1	0	2	8.3	.117	8.4	6.3	1	1	0	2	8.0	.113	7.6	6.6	1	1	0	2	8.0	.104	7.0	8.1	1	1	0	2	8.0	.106	7.8	9.8	1	1	0	2	9.0	.115	6.1	5.6
0	1	1	2	7.7	.104	8.8	6.0	0	1	1	2	6.3	.101	10.2	5.1	0	1	1	2	8.3	.113	7.9	7.1	0	1	1	2	8.0	.125	8.8	9.1	0	1	1	2	9.7	.111	7.1	8.8
1	0	1	2	9.7	.126	8.8	6.2	1	0	1	2	6.0	.081	8.4	6.4	1	0	1	2	8.3	.133	8.1	8.2	1	0	1	2	7.3	.087	7.4	8.8	0	1	0	2	8.7	.114	6.1	7.6
2	0	0	2	8.3	.126	8.7	5.3	2	0	0	2	9.7	.112	7.1	6.6	2	0	0	2	9.0	.108	6.7	9.7	2	0	0	2	7.0	.083	7.0	9.3	2	0	0	2	8.7	.113	5.7	8.9
0	2	0	2	7.0	.098	9.0	5.6	0	2	0	2	8.3	.099	6.9	7.4	0	2	0	2	8.0	.096	5.5	8.0	0	2	0	2	9.0	.135	7.8	8.5	0	2	0	2	8.3	.103	7.0	6.7
0	0	2	2	8.7	.124	8.8	6.0	0	0	2	2	5.7	.092	8.9	6.0	0	0	2	2	8.7	.136	9.2	7.2	0	0	2	2	7.3	.100	8.2	9.3	0	0	2	2	7.0	.066	4.4	11.5
1	1	2	4	8.3	.150	9.5	6.2	1	1	2	4	6.7	.096	8.9	5.6	1	1	2	4	8.7	.093	5.3	10.5	1	1	2	4	6.0	.063	4.9	13.7	1	1	2	4	5.7	.050	3.8	11.9
2	1	1	4	9.7	.130	8.0	7.9	2	1	1	4	5.7	.073	8.2	6.2	2	1	1	4	7.0	.063	4.1	13.1	2	1	1	4	5.7	.048	3.6	14.7	2	1	1	4	7.7	.095	4.6	11.1
1	2	1	4	9.3	.124	8.5	6.2	1	2	1	4	5.7	.088	9.1	5.2	1	2	1	4	7.0	.095	5.4	10.9	1	2	1	4	4.0	.029	3.4	14.8	1	2	1	4	6.3	.091	5.2	10.4
1	3	0	4	8.3	.107	7.3	6.6	1	3	0	4	5.0	.084	9.0	5.7	1	3	0	4	7.0	.066	2.8	11.8	1	3	0	4	6.0	.033	2.7	14.2	1	3	0	4	5.3	.068	3.9	9.5
0	1	3	4	8.0	.115	7.7	6.2	0	1	3	4	6.3	.095	9.3	6.6	0	1	3	4	8.3	.104	5.7	9.8	0	1	3	4	7.0	.063	4.1	13.8	0	1	3	4	1.3	.008	2.7	14.0
0	3	1	4	8.0	.114	8.0	7.2	0	3	1	4	6.0	.098	9.6	5.4	0	3	1	4	7.0	.071	3.2	11.5	0	3	1	4	4.7	.032	3.8	14.7	0	3	1	4	7.0	.089	5.7	11.6
1	0	3	4	7.3	.112	9.2	6.2	1	0	3	4	6.3	.090	8.7	6.0	1	0	3	4	6.7	.079	5.5	11.4	1	0	3	4	4.7	.046	4.3	13.4	1	0	3	4	5.3	.027	2.6	14.0
3	1	0	4	7.7	.098	7.0	7.7	3	1	0	4	6.7	.099	7.0	7.6	3	1	0	4	7.0	.044	3.1	13.2	3	1	0	4	5.7	.030	2.2	13.0	3	1	0	4	8.3	.107	4.8	9.1
3	0	1	4	9.0	.110	7.6	7.7	3	0	1	4	6.7	.113	10.2	5.4	3	0	1	4	6.0	.057	3.9	14.1	3	0	1	4	5.3	.039	2.9	13.2	3	0	1	4	7.7	.061	3.6	12.4
2	2	0	4	7.7	.088	7.3	7.6	2	2	0	4	6.0	.091	7.4	7.3	2	2	0	4	4.0	.032	3.7	11.3	2	2	0	4	5.0	.030	3.7	14.8	2	2	0	4	7.7	.091	3.7	8.0
0	2	2	4	9.3	.149	8.6	6.1	0	2	2	4	6.7	.095	8.5	5.8	0	2	2	4	8.3	.099	5.2	10.8	0	2	2	4	6.0	.058	5.1	14.3	0	2	2	4	6.0	.051	5.1	14.0
2	0	2	4	9.0	.114	8.1	6.6	2	0	2	4	6.0	.092	9.8	5.3	2	0	2	4	6.3	.066	4.5	11.7	2	0	2	4	6.0	.057	4.5	13.0	2	0	2	4	4.7	.034	2.2	13.7
4	0	0	4	7.7	.103	7.9	7.8	4	0	0	4	6.3	.089	6.7	7.2	4	0	0	4	5.3	.051	3.7	13.5	4	0	0	4	5.7	.033	3.1	13.2	4	0	0	4	9.3	.119	5.1	8.0
0	4	0	4	8.7	.115	7.2	7.2	0	4	0	4	5.7	.083	8.9	5.2	0	4	0	4	6.7	.052	2.0	12.8	0	4	0	4	6.3	.064	5.3	14.1	0	4	0	4	6.3	.081	6.0	7.8
0	0	4	4	6.7	.071	7.4	6.2	0	0	4	4	6.3	.066	6.2	6.6	0	0	4	4	8.0	.093	5.3	10.8	0	0	4	4	6.3	.056	4.9	13.0	0	0	4	4	1.7	.009	3.0	14.0

| | | |

The top line in each case gives the results where no salts were applied. Below this the figures are arranged according to the total quantity of salt used, first 1,000 p. p. m., followed by 2,000, 4,000, 6,000, 8,000, and 10,000. It will be noted that with the chlorids and nitrates practically no plants grew in the higher concentrations. Careful study of the table is necessary to see the numerous complex relations that are brought out between the various salts. The simple relations may be seen more easily in figures 2 to 24, but by bringing together a large mass of data in one table many relations can be found that could not be seen in the diagrams.

The average alkali of Utah contains a mixture of chlorids, sulphates, and carbonates, with the carbonates usually present only in small quantities. The practical alkali problem, therefore, is largely centered around the sulphates and chlorids of sodium. An examination of Table XVI does not seem to indicate that either of these salts has any great neutralizing effect on the other.

A general conclusion from this table might be that where alkali salts are found together in the soil the toxic action of the combined salts is only slightly less than the sum of the toxicities of the individual salts. It may be that with other combinations of salts this conclusion would not be justified.

PRACTICAL LIMITS OF THIS PROBLEM

The practical problem of this entire study is to determine the quantity of various alkali salts necessary in the soil to reduce the growth of crops beyond the point of profitable production. Under the conditions of dry farming there is no practicable way of removing excessive soluble salts; hence, if salts are found in these soils in quantities prohibiting crop growth, the soils are valueless for agriculture. On the other hand, soils that are susceptible of irrigation and drainage may be reclaimed by the leaching out of the alkali. In any soil, however, where there is a likelihood of alkali injury it is very important, in order to be able to judge the value of a soil, to know exactly how much of a given salt is necessary to injure crops. The literature on the subject up to the present is somewhat conflicting and lacks the definiteness that would be desirable.

There are so many factors entering into the toxicity of alkali that it is difficult to assign definite toxic limits. For example, an analysis might show a soil to contain a given percentage of salt when in reality the greater part of the salt might be in a crystallized form at the surface, where it would do no harm until dissolved and washed back into the soil. It is the salt in solution that does the real injury. The wetness of the soil, its texture, the presence of neutralizing substances, and a number of other factors all alter the toxicity of soluble salts, which

makes it impossible to say exactly what are the practical limits of alkalies.

In getting the limits given below it was considered that when alkali retarded germination and growth to about half what they were in soils without alkali the practical limit had been reached. Certainly it would not be profitable to use a soil where alkali decreased yields below half normal.

Figures 46 and 47 show the practical limits of growth of wheat in loam and sand for 13 different salts. It will be noted that these salts bear a similar relation to each other in both kinds of soil, although only about half as much alkali is required in sand to reach the toxic limit as in loam. One of the most striking features about the diagram is the fact that in sand the carbonates are proportionately more toxic when compared with other salts than they are in loam.

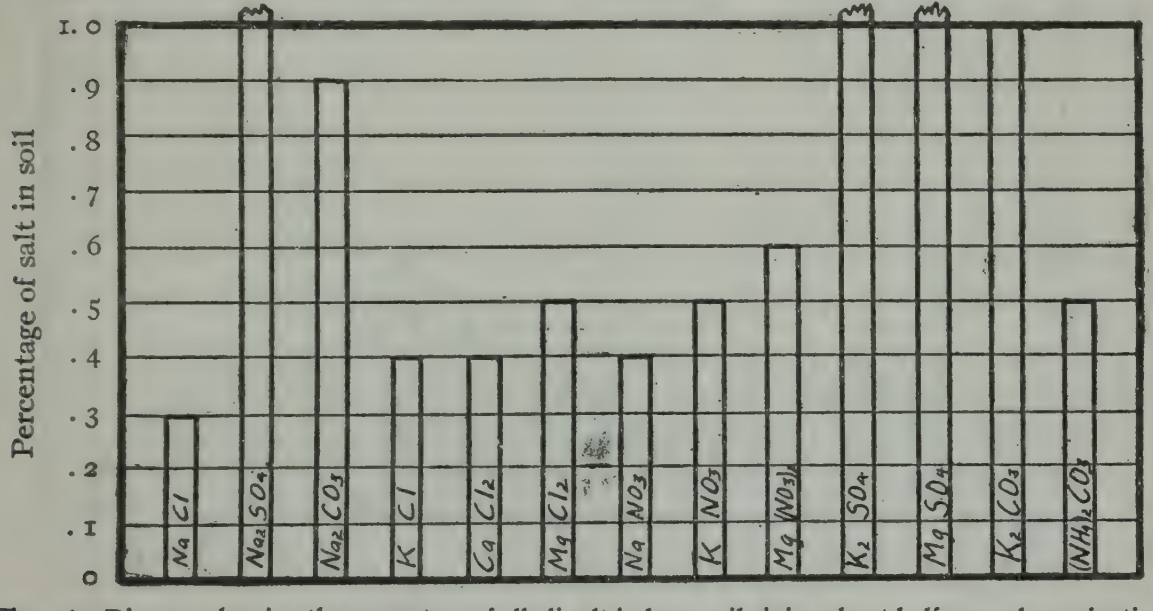

FIG. 46.—Diagram showing the percentage of alkali salt in loam soil giving about half normal germination and production of dry matter in wheat.

Loam having 0.3 per cent and sand having 0.2 per cent of sodium chlorid contain a limit of this salt for the profitable production of crops. The other chlorids may be somewhat higher, while the nitrates may be about 0.1 per cent higher than the chlorids. On loam crops grow well with as high as 1 per cent of the sulphates, while in sand from 0.5 to 0.7 per cent of the sulphates is injurious.

Figure 48 gives a comparison of the resistance of barley, oats, wheat, alfalfa, sugar beets, corn, and Canada field peas for sodium chlorid, sodium carbonate, and sodium sulphate in loam. Barley can withstand 0.5 per cent of sodium chlorid, 1 per cent of sodium carbonate, and more than 1 per cent of sodium sulphate. All crops in the test except oats, sugar beets, corn, and field peas produced more than half normal growth where 1 per cent of sodium sulphate was present. There was a great difference in the resistance of various crops to sodium carbonate, the

practical limit ranging from 0.4 per cent for Canada field peas up to 1 per cent for barley. Sodium chlorid showed about the same toxicity for all the crops except barley and oats, which were slightly more resistant. The striking point about this diagram is the fact that the relative toxicity of the different salts varies for each crop.

SUMMARY

(1) The effect of the various alkali salts in soils on plant growth and the quantity of alkali that must be present to injure crops are of great practical importance to farmers in arid regions, as well as of considerable interest to the scientist.

(2) A great amount of work has already been done on alkali, but this does not give all the information that is needed.

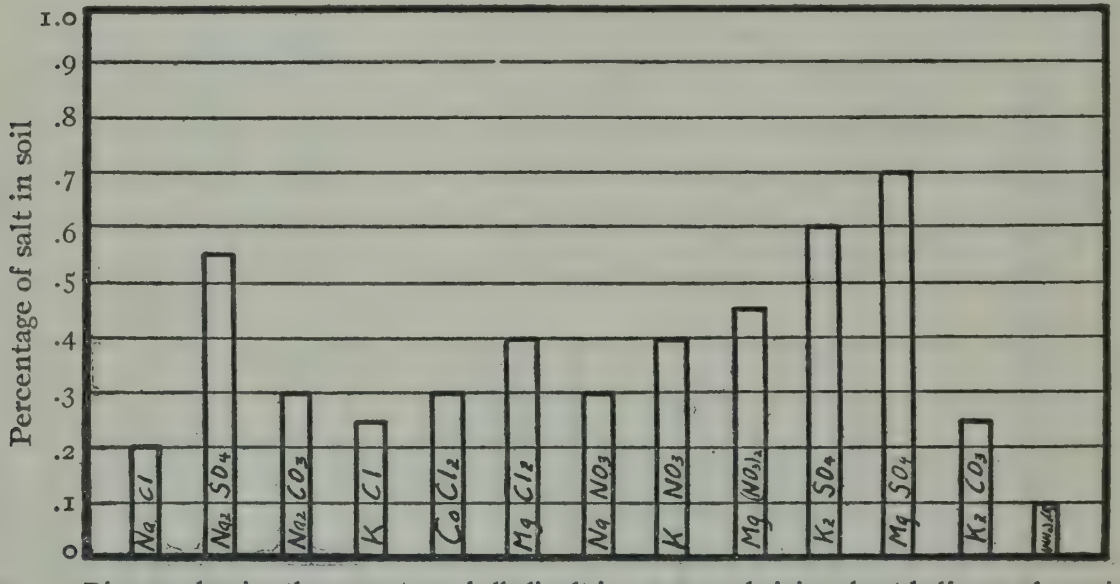

FIG. 47.—Diagram showing the percentage of alkali salt in coarse sand giving about half normal germination and production of dry matter in wheat.

(3) In this paper results of over 18,000 determinations of the effect of alkali salts on plant growth are reported.

(4) Only about half as much alkali is required to prohibit the growth of crops in sand as in loam.

(5) Crops vary greatly in their relative resistance to alkali salts, but for the ordinary mixture of salts the following crops in the seedling stage would probably come in the order given, barley being the most resistant: Barley, oats, wheat, alfalfa, sugar beets, corn, and Canada field peas.

(6) Results obtained in solution cultures for the toxicity of alkali salts do not always hold when these salts are applied to the soil.

(7) The percentage of germination of seeds, the quantity of dry matter produced, the height of plants, and the number of leaves per plant are all affected by alkali salts in about the same ratio.

(8) The period of germination of seeds is considerably lengthened by the presence of soluble salts in the soil.

(9) The anion, or acid radical, and not the cation, or basic radical, determines the toxicity of alkali salts in the soil. Of the acid radicals used, chlorid was decidedly the most toxic, while sodium was the most toxic base.

(10) The injurious action of alkali salts is not in all cases proportional to the osmotic pressure of the salts.

(11) The toxicity of soluble salts in the soil was found to be in the following order: Sodium chlorid, calcium chlorid, potassium chlorid, sodium nitrate, magnesium chlorid, potassium nitrate, magnesium nitrate,

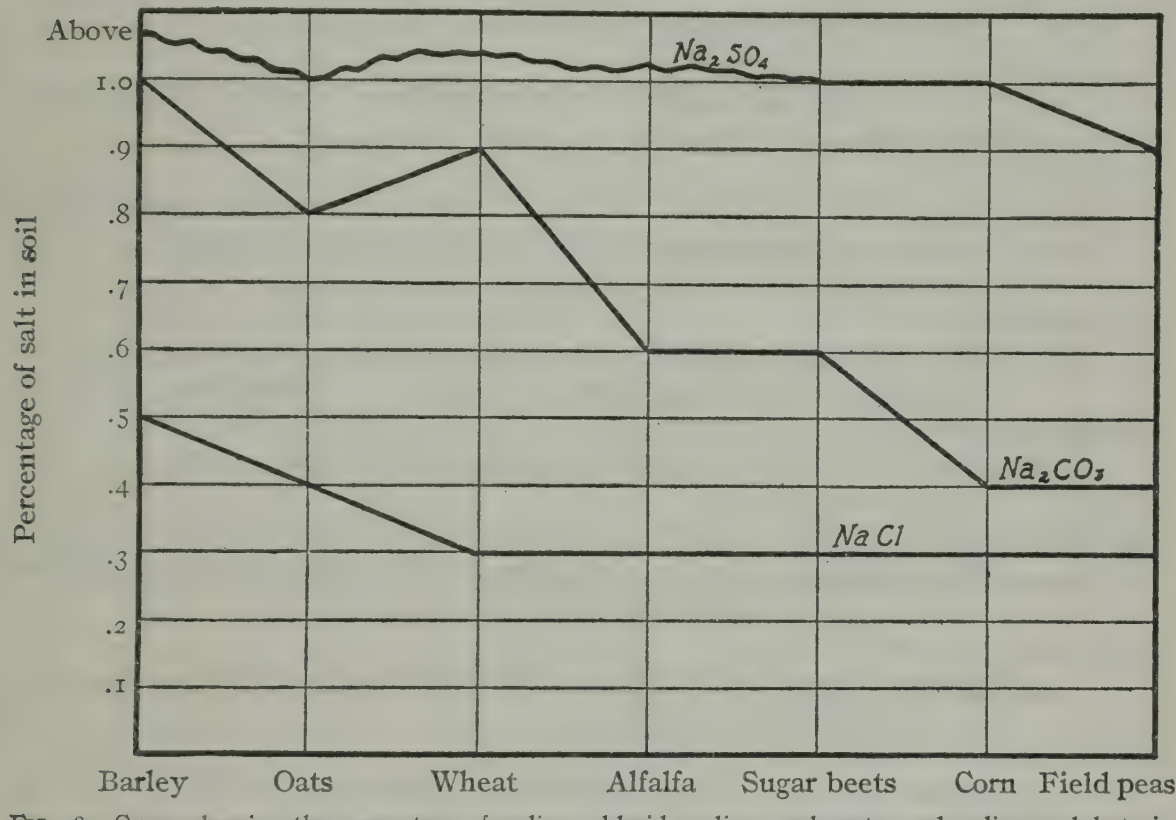

FIG. 48.—Curve showing the percentage of sodium chlorid, sodium carbonate, and sodium sulphate in Greenville loam giving about half normal germination and production of dry matter.

sodium carbonate, potassium carbonate, sodium sulphate, potassium sulphate, and magnesium sulphate.

(12) The antagonistic effect of combined salts was not so great in soils as in solution cultures.

(13) The percentage of soil moisture influences the toxicity of alkali salts.

(14) Salts added to the soil in the dry state do not have so great an effect as those added in solution.

(15) Land containing more than about the following percentages of soluble salt are probably not suited without reclamation to produce ordinary crops. In loam, chlorids, 0.3 per cent; nitrates, 0.4 per cent; carbonates, 0.5 per cent; sulphates, above 1.0 per cent. In coarse sand, chlorids, 0.2 per cent; nitrates, 0.3 per cent; carbonates, 0.3 per cent; and sulphates, 0.6 per cent.

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HISTOLOGICAL RELATIONS OF SUGAR-BEET SEEDLINGS AND PHOMA BETAE

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In a former paper [2] it was pointed out that practically all sugar-beet (*Beta vulgaris*) seed is more or less heavily infected with *Phoma betae* (Oud.) Fr., and that a large proportion of the seedlings developing from such stock suffer from incipient or severe attack of the fungus, but that under favorable conditions a high percentage of the attacked plants recover sufficiently to make a good growth. It appears that the period during which the sugar beet is susceptible to infection by this fungus is confined to the seedling stage, or, in the case of leaves, to old age, but that when infection has once occurred, it persists. After apparent recovery of the host, the fungus is still present, although it remains concealed until conditions arise sufficiently unfavorable to the beet to enable the parasite to renew its attack. Except in the seedling stage, it seldom accomplishes the immediate destruction of its host, but remains inactive during the first growing season and becomes destructive on mother beets in storage or reappears during the second growing season on the seed stalks or racemes in time to cause infection of the new crop of seed.

Histological studies recently conducted upon seedling sugar beets infected with *Phoma betae* have shown the fungus fruiting on the surface of young plants that were scarcely past the cotyledon stage. They have also revealed the organism living without serious injury to the host, within the deeper cells of plants that had thrown off the attack and which could safely be predicted to show no further sign of infection during the growing season if reasonably good cultural conditions were maintained. The slides show that the fungus may persist both in and on the tissues of the beet and also indicate something of its *modus operandi* in attack on seedlings. Sections were prepared from material grown from pasteurized seed in experimental pots in sterilized soil which had been inoculated at the time of seeding with pure cultures of the fungus. The material was controlled by check pots and by recovery of the fungus from certain of the seedlings from each pot as the disease appeared. Damped-off and root-sick seedlings selected at different stages in the progress of the disease and healthy

[1] The author wishes to acknowledge his indebtedness to Mrs. Nellie D. Morey, formerly of the Office of Cotton and Truck Disease Investigations, for assistance in the preparation of slides.

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Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C
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Vol. V, No. 1
Oct. 4, 1915
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seedlings from the control pots were killed in Flemming's solution, embedded, sectioned, and stained with the triple combination in the usual way. Camera-lucida drawings from the slides thus prepared are employed to illustrate this discussion. Most of the seedlings were still in the cotyledon stage, but some that had recovered from the attack had developed their first pairs of leaves. Seedlings which had been entirely killed were so badly disintegrated or so softened by the disease that they did not yield satisfactory material for study. The sections showed the cells in a condition of complete collapse and decay. The cellulose layers of the walls, as well as the middle lamella, were gelatinized and softened to such an extent as to have lost most of their rigidity. The walls were broken and fragmented, but this may have resulted from handling during the process of washing and dehydrating. Bacteria were present, of course, and the softening of the walls, which made them so liable to fracture in handling, may have been due in part to the action of these agents.

Cells of badly diseased but still living seedlings presented more favorable material for studying the histological relations of the parasite and host. The cells were often nearly filled with the fungus, which showed a tendency to remain within the cell rather than in the middle lamella, although it frequently penetrated the walls (Pl. I, fig. 1). Now and then a thread of the fungus was observed running between the cells for a little distance, but the indications are that, while the organism dissolves the middle lamella, it does not feed upon it. Heavily invaded cells are consumed, the cytoplasm disappears, and the nuclei disintegrate. The middle lamella gelatinizes, so that the cellulose lamellæ may become widely separated while the cellulose layers are broken and disintegrated or even dissolved (Pl. I, fig. 2). The first visible indication of the alteration in the walls is a change in their reaction toward the stain. They take the safranine more deeply and retain it more tenaciously than do the walls of normal cells. With the progress of the disease a border area of increasing width, which also takes the safranine deeply, develops on either side of the walls, as if the substances which retained the dye were gradually diffusing from the wall and spreading into the surrounding space.

In cases of less serious infection, where recovery is possible, or in tissues which have just been invaded, a somewhat different condition exists. Plate I, figure 3, represents a recently invaded portion of a rather badly diseased seedling which would probably have been unable to recover. The cell walls show the gelatinized condition only in a moderate degree and in an area confined to the points where it has been penetrated by the mycelium. The mycelium has expanded in one of the cells in a manner not frequently noted, and the effect of the parasitism is apparent in the abnormal condition of the host nuclei. Evidence of disease was sometimes manifested in the neighboring uninfected cells of such mate-

rial by the unusual appearance of the nuclei. Dumb-bell forms, budding, and indirect division were observed occasionally, but never in any large number (Pl. I, fig. 4, 5, 6).

The most interesting phenomena in many respects, as well as the most puzzling, are those associated with recovery and healing. Sugar beets attacked by the fungus frequently send out new side roots from a point above the invasion and succeed in preventing the destruction of this new growth. Cases were common in which the region invaded and disintegrated had been confined to the outer tissue. The central vascular region and the surrounding layers of cells resisted the attack and eventually succeeded in sloughing off the killed tissue. The fungus was frequently found developing its pycnidia on the killed portions of such recovering seedlings, while the host tissue, only a few cells below, appeared perfectly normal (Pl. II, fig. 1).

The most striking thing brought out by a study of the sections, however, is the presence of the fungus apparently established in a condition of reduced relative virulence in the interior tissue of beets which have recovered from the attack and which are assured of making a good growth (Pl. II, fig. 2). In such cases even the invaded cells are not killed, and the adjacent ones appear perfectly normal in every respect. So far as has been observed, the cells thus invaded are adjacent to vascular tissue, but the organism has never been seen in the conducting elements. The infection is confined to a vertical chain of cells, and in no case was more than a single unbranched hypha observed.

The physiological relation here presented is an exceedingly interesting one and its investigation is of the highest scientific and practical importance.

It is difficult to explain just how an organism capable of producing such complete collapse in cells of seedlings should suddenly find its action checked and confined to a saprophytic existence on an area of discarded surface tissue, but the means by which it establishes itself within the highly nutritive living cells of the interior and is at the same time compelled to remain in a quiescent condition is still more problematical. The condition presents a relatively highly developed type of parasitism in which the organism voluntarily or by compulsion permits the completion of the normal life history of the host while securing for itself the assurance of perpetuation through infection of the seed. The balance, however, is not a perfect one, since, if the host encounters sufficiently adverse conditions during either of the growing seasons or in storage, the activity of the parasite is renewed and the sugar beet is destroyed, thus preventing seed production and the perpetuation of the parasite through the seedling channel.

PLATE I

Fig. 1.—Section of a sugar-beet seedling invaded by *Phoma betae*, showing distribution of the mycelium and the action of the fungus on the protoplasm and cell walls. × 530.

Fig. 2.—Section of sugar-beet seedling showing characteristic action of *Phoma betae* on the cytoplasm and nuclei and cell walls in cases of serious infection. Note the gelatinized condition of the middle lamella. × 530.

Fig. 3.—Section of sugar-beet seedling showing *Phoma betae* penetrating the cell walls and expanding in one of the cells. The nuclei show signs of degeneration. × 530.

Fig. 4, 5, and 6.—Abnormal nuclei from uninfected cells adjacent to invaded tissue of sugar-beet seedlings. The nucleus in figure 6 appears to be in the process of direct division. × 1,330.

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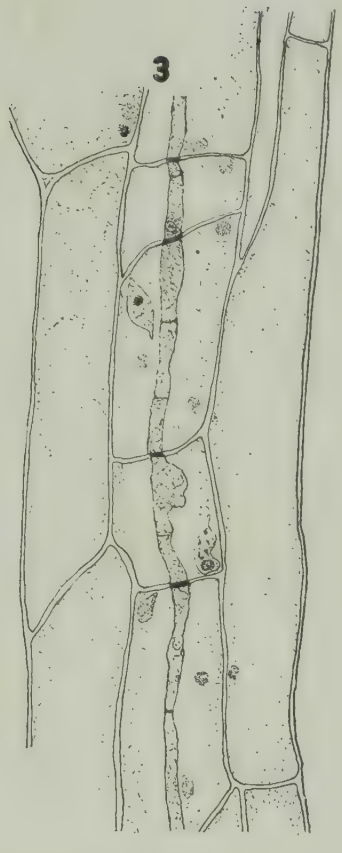
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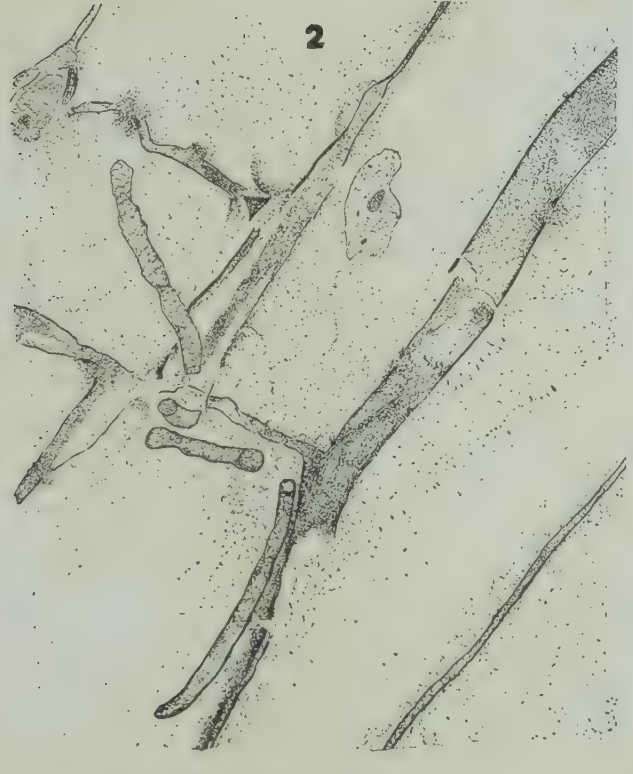
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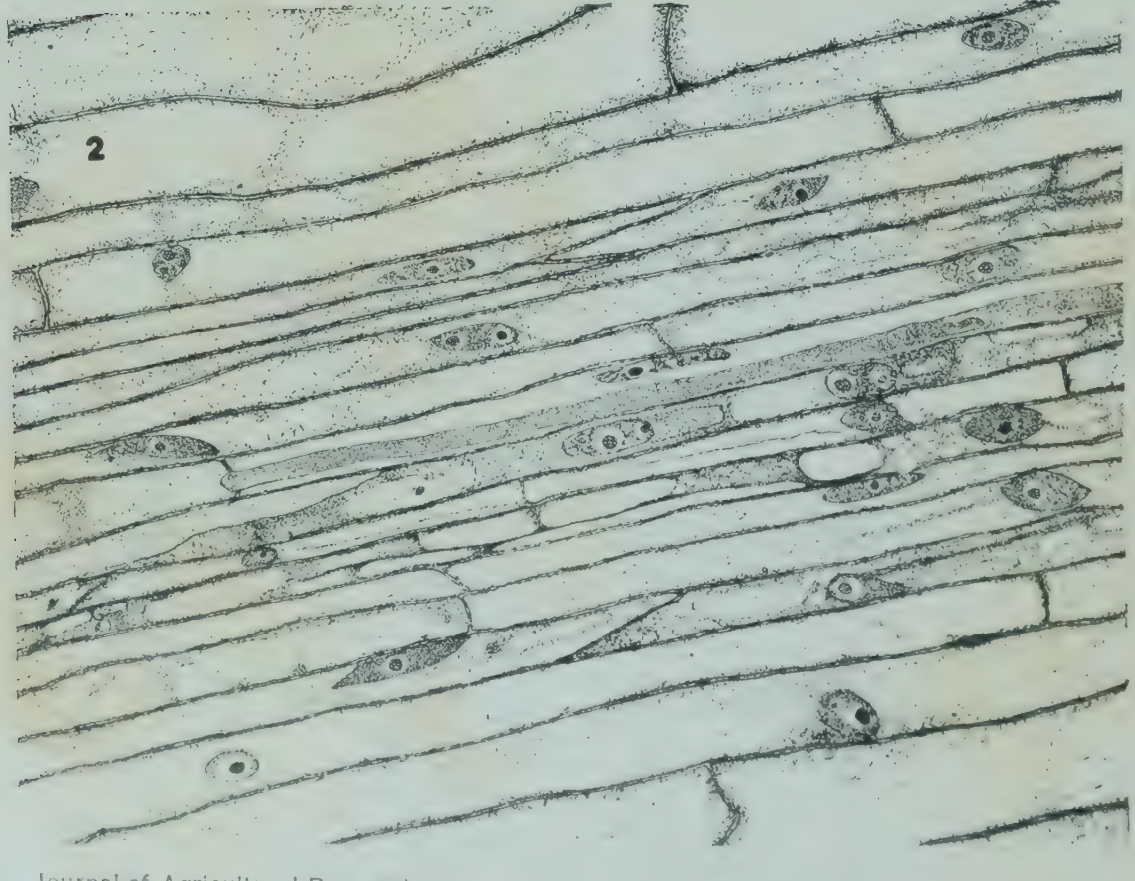
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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., OCTOBER 11, 1915 NO. 2

PERENNIAL MYCELIUM IN SPECIES OF PERONOSPORACEAE RELATED TO PHYTOPHTHORA INFESTANS

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INTRODUCTION

Phytophthora infestans having been found to be perennial in the Irish potato (*Solanum tuberosum*), the question naturally arose as to whether other species of Peronosporaceae survive the winter in the northern part of the United States in the mycelial stage. As shown in another paper (13),[1] the mycelium in the mother tuber grows up the stem to the surface of the soil and causes an infection of the foliage which may result in an epidemic of late-blight.

Very little is known about the perennial nature of the mycelium of Peronosporaceae. Only two species have been reported in America: *Plasmopara pygmaea* on *Hepatica acutiloba* by Stewart (15) and *Phytophthora cactorum* on *Panax quinquefolium* by Rosenbaum (14). Six have been shown to be perennial in Europe: *Peronospora schachtii* on *Beta vulgaris* and *Peronospora dipsaci* on *Dipsacus fullonum* by Kühn (7, 8); *Peronospora alsinearum* on *Stellaria media*, *Peronospora grisea* on *Veronica hederaefolia*, *Peronospora effusa* on *Spinacia oleracea*, and *Atriplex hortensis* by Magnus (9); and *Peronospora viticola* on *Vitis vinifera* by Istvanffi (5).

Many of the hosts of this family are annuals, but some are biennials, or, like the Irish potato, are perennials. Where the host lives over the winter, it is interesting to know whether the mycelium of the fungus may also live over, especially where the infection has become systemic and the mycelium is present in the crown of the host plant. The absence or sparse production of oospores in some of the species of Peronosporaceae, coupled with the appearance of the fungus as soon as the host puts out foliage in the spring, suggests that the mycelium may play an important

[1] Reference is made by number to "Literature cited," p. 68–69.

rôle in bridging over the winter. This paper gives the results of experiments and observations which show that in the Northern States species of the Peronosporaceae which have perennial mycelium are common and that the mycelium may live from one growing season to another in the living diseased host tissues.

In several of these experiments the locality where infected plants were growing was marked in the autumn and the plants collected from time to time during the winter and early spring, after which they were allowed to revive in the greenhouse and a careful watch kept for any evidence of fruit of the fungus. In other cases the underground parts of infected plants were taken in the spring and planted in steam-sterilized soil in the greenhouse, and when the shoots came through the ground conditions were made favorable for the sporulation of the fungus. In still other cases the presence of the mycelium in perennial parts of the host was determined microscopically.

PERONOSPORA PARASITICA

Late in the fall of 1910 and 1911 it was observed that young plants of *Lepidium virginicum* in the vicinity of Madison, Wis., were very generally infected with *Peronospora parasitica* and that the tissues of these plants contained few or no oospores, although they were produced in abundance in the summer when the host tissues were dying. Plants of *Lepidium* sp. always form a rosette of leaves in the late fall, and some of these remain alive through the winter.

In the fall of 1911 two patches of Lepidium plants, about 50 per cent of which were infected with *Peronospora parasitica*, were marked so that they might be easily found during the winter. One was on the side of a short incline made by dumping several loads of soil in a heap and the other on the parking of a city drive in Madison. Both patches were well exposed during the winter of 1911–12, which was unusually severe, there being no covering of snow on the former at any time and the latter being covered only a part of the time.

After the first killing frost, which, according to the Weather Bureau, occurred on October 24, infected plants of *Lepidium virginicum* were collected at various times during the winter. Beginning on October 30, a test was made of the germination of the conidia of *Peronospora parasitica* growing on *Lepidium virginicum*. Although when alive the conidia of this fungus usually germinate profusely within 2 to 3 hours and always within 24 hours, no germination occurred in this test, although exposed to favorable conditions for 48 hours. This coincides with what is known of the behavior of the spores of other species—e. g., *Cystopus candidus* (Melhus, 10)—and excludes the possibility of these conidia becoming a source of further infection. A careful search for oospores was made after October 30 in a large number of infected plantlets, but none was found.

The first collection of plants of *Lepidium virginicum*, numbering about 20, was made on November 5, enough soil being taken up with each plant to keep the roots from being disturbed. The plants were taken to the greenhouse and transplanted in two flats, or shallow boxes, and on November 6 each box was covered with a low bell jar to keep the air moist, a condition favorable for the sporulation of the fungus. An examination of the plants next day showed but 2 inactive, the leaves of the other 18 being turgid and expanded in the normal way. It also showed that 2 of the plants were covered with a white glistening growth, which on microscopic examination was found to be the spores and conidiophores of *Peronospora parasitica*. The following day this fungus was found sporulating on 3 additional plants, and 8 days after the plants had been collected it was found fruiting on some portion of 12 of the 18 living. Although kept under observation for 6 weeks, the remaining six plants were free from infection, which showed that it did not take place under the conditions in which they were held in the greenhouse.

On December 14 another collection of plants of *Lepidium virginicum* was taken from the patch on the parking near the drive, the soil at that time being frozen 6 inches deep. A block of soil on which there were 18 of the plants was chopped loose and placed in a flat in the greenhouse, and after being allowed to thaw out for 24 hours was covered with a glass house. On December 17, 3 days after the plants were brought into the greenhouse, 1 was nearly covered with conidiophores and spores of *Peronospora parasitica*, the next day 4 more showed fruit of the fungus, and at the end of the sixth day an additional plant, or 6 in all, showed fruiting of the fungus, indicating that at least that number was infected when collected (Pl. III, fig. 2, *A*). The fungus fruited on both sides of the leaves and also on the new leaves developing from the crown, though not as abundantly on these as on the older leaves.

Besides the collections of November 5 and December 14, 4 others, or a total of 102 plants, were brought into the greenhouse from the 2 patches during the dormant period of the host plant. In the case of several of these collections *Peronospora parasitica* sporulated on some of the plants 2 days after their transfer to the greenhouse, but usually the disease did not appear before 3 to 5 days and, when the infection was weak, not before 8 days after the transfer. Table I gives date of collection, number of plants in each collection, date of first evidence of Peronospora, number of days required for the fungus to sporulate, and number of plants on which the disease appeared.

TABLE I.—*Record of six collections of plants of Lepidium virginicum infected with Peronospora parasitica*

Date of collection.	Number of plants.	Date of sporulation.	Number of days required for sporulation.	Number of plants on which fungus sporulated.
1911.				
Nov. 5	20	Nov. 8	3	12
Dec. 14	18	Dec. 17	3	6
Dec. 18	12	Dec. 20	2	6
1912.				
Feb. 22	11	Feb. 27	5	1
Mar. 6	24	Mar. 10	4	7
Mar. 25	17	Mar. 27	2	9

As shown by Table I, 41 plants, or about 40 per cent of the collections, were infected before their transfer to the greenhouse.

It might be supposed that oospores produced the previous year were in the soil immediately around and adhering to the plants collected and that when warmed up in the greenhouse these germinated and produced the infections noted. To test this possibility, 25 leaves were collected from the plants in the two patches, washed very thoroughly in running water, and placed in a moist chamber, while 25 other leaves were collected from the same plants, and without being washed were placed under similar conditions as controls. In both cases the fungus sporulated after three days, and, although much less than when the leaves were on the plant, the sporulation produced sufficient conidiophores to be plainly visible to the naked eye, a growth which could probably not be produced by oospores.

Besides this evidence that *Peronospora parasitica* renews itself by means of mycelium as well as oospores, the writer failed to germinate oospores after repeated attempts. He has also shown (11) that *Peronospora parasitica* on *Lepidium virginicum* can be collected at any time during the winter and early spring, brought into the greenhouse, and made to fruit. Moreover, there can be no doubt that the sporulation on the plant collections at Madison was due to living mycelium in the host tissue.

CYSTOPUS CANDIDUS

Lepidium virginicum is attacked not only by *Peronospora parasitica* but also by *Cystopus candidus*, a fungus which can undoubtedly propagate itself from year to year by mycelium remaining dormant in the living host tissues through the winter. As is well known, these two fungi often infect a plant simultaneously, as was the case of some of the plants from the parking near the drive. In the collections made on December 14, 1911, one plant showed white pustules of *Cystopus candidus* on December 17, three days after the plants were collected. The following day

two additional plants showed white pustules of this fungus and also spores of *Peronospora parasitica*, the number of pustules increasing on the lower side of the leaves until many were well spotted. Two plants in the collection made on February 22 bore white pustules within three days after they were taken into the greenhouse, showing that they were infected with *Cystopus candidus* and that the fungus was alive in the tissues in late winter (Pl. III, fig. 1). Again, in the collection made on March 25 one plant developed pustules of *Cystopus candidus* and conidiophores and spores of *Peronospora parasitica* four days after being transferred to the greenhouse.

Cystopus candidus is also a very common parasite on *Capsella bursa pastoris*, a plant that may become a winter annual. In the fall of 1911 a patch of plants of *Capsella bursa pastoris*, many of which were infected with *Cystopus candidus*, was marked; and on March 30, 1912, 25 plants were collected and treated in the same way as the plants of *Lepidium virginicum* infected with *Peronospora parasitica*. After two days the plants began to show signs of life; and at this time they were covered with a small glass house. Three days later white pustules were discovered on one leaf; and the following day, or six days after the plants were brought in, white pustules developed on other leaves of the same plant.

On April 5, 1912, just as the ground thawed out, another collection, consisting of 76 plants, was made. Four days after, or on April 9, there were white pustules on four of the plants. Except in the case of one large leaf, which was probably produced early the preceding fall, the pustules were all on the youngest leaves, which indicates that the mycelium can winter over in leaves of plants of *Capsella bursa pastoris* that live through the winter. The fact that the youngest leaves were infected suggested crown infection; and later this proved to be the case, all of the leaves growing from certain plants being infected as soon as they appeared, while the leaves growing from certain others remained free from infection. On April 10 white pustules appeared on two other plants, making a total of six infected plants in the second collection. As soon as the plants of *Capsella bursa pastoris* in the marked patch started to grow in the spring some of them showed infection with *Cystopus candidus*, which developed like the infections studied in the greenhouse. From these experiments it will be seen that the mycelium of *Cystopus candidus* in the tissues of the host remains alive through the winter.

In the fall of the year *Cystopus candidus* becomes systemic in the tissues of *Sisymbrium officinale* and *Brassica nigra* also. So far these two host plants have not been followed through from fall to spring, but, like the plants of *Lepidium virginicum* and *Capsula bursa pastoris*, both may become winter annuals, as is well known.

PERONOSPORA FICARIAE

On May 10, 1911, *Peronospora ficariae* was very prevalent on *Ranunculus fasciculatis* in the vicinity of Madison. This fact, coupled with De Bary's (3) statement in connection with his discussion of the perennial nature of mycelium of *Phytophthora infestans*, that *Peronospora ficariae* is perennial in the tissues of *Ranunculus ficaria*, led the writer to determine whether it survives the winter in the mycelial stage on *Ranunculus fasciculatis* also. Eighteen plants, very generally infected with the disease, were staked on the date above mentioned so that they could be readily located throughout the winter and following spring. On February 2, 1912, five of the plants were chopped out of the frozen ground and carried into the greenhouse, where the adhering soil was allowed to thaw out and was removed from the fascicled roots, after which the roots were carefully washed until free from soil and then transplanted in greenhouse soil. The plants, two of which refused to grow, started very slowly, the first one coming up on March 3, and two others the following day. The young plants were chlorotic, distorted, and yellowish green, but there was no evidence of *Peronospora ficariae* present until they had been held under small bell jars for 24 hours, after which the fungus present on the deformed leaves fruited profusely, showing plainly that the fungus was alive in the host tissues during the winter.

The 13 plants that were left in the marked space from which the 5 were taken were also watched carefully after they began to come up. On April 5 five appeared, and these were covered with small bell jars. On the following day conidiophores and spores of *Peronospora ficariae* were collected from the underside of the leaves, showing that in this case also the plants were infected before they reached the surface of the soil. The results of these experiments confirm De Bary's (3) statement and also show that *Peronospora ficariae* is perennial not only in *Ranunculus ficaria* but also in *Ranunculus fasciculatis*.

PERONOSPORA VICIAE

Peronospora viciae occurs on several of the legumes. On May 11, 1913, the writer found it to be quite abundant on *Vicia sepium*, a perennial common in the District of Columbia. At that time about 25 per cent of the plants, which were from 4 to 6 inches high, were infected with the disease, the fungus sporulating profusely and the plants giving every evidence of systemic infection. The location of these plants was staked off on the date above mentioned and the patch kept under observation. On April 5 the following spring the plants started to come up, the tallest being only 2 inches, and at this early stage nine were found to be systemically infected. It was not uncommon to find a healthy and a diseased plant within 2 inches of each other. If infection was caused by oospores or conidia, it is difficult to understand why the infection was not general

in the patch and why plants growing near each other should be infected in some cases and not in others.

As the host is a perennial, as infection by *Peronospora viciae* is systemic, and as oospores are produced only sparingly, if at all, on *Vicia sepium*,[1] it seems very probable that the mycelium survives the winter in the living tissues of the host.

PLASMOPARA HALSTEDII

In the spring of 1911 *Plasmopara halstedii* was found to be very abundant on some young plants of *Helianthus diversicatus* about 6 inches high. The plants were somewhat dwarfed, chlorotic, and well covered with conidiophores, giving every evidence of systemic infection. The location of the infected plants was marked and observations made during the winter and spring of 1912.

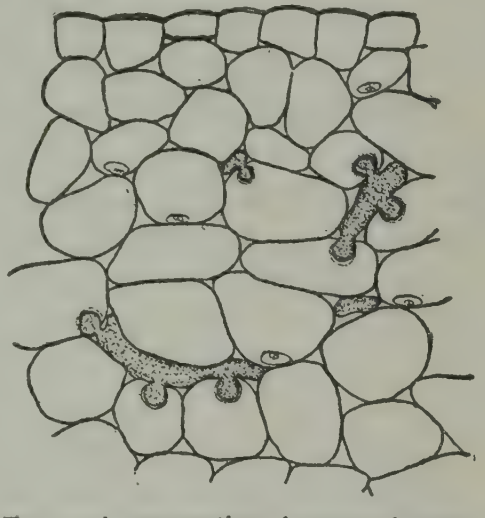

FIG. 1.—A cross section of a stem of *Helianthus diversicatus* which is infected with *Plasmopara halstedii*. The mycelium is shown in the cortex at the junction of the stem with the rhizome of the host.

Fourteen of the plants that were very generally infected were staked, and on January 4, three of these were chopped out of the ground and transplanted in the greenhouse in exactly the same way as were the Lepidium plants infected with *Peronospora parasitica*. Each of these rhizomes produced a chlorotic shoot which was covered with spores of *Plasmopara halstedii*. On March 4 four more were brought into the greenhouse. One of these rotted in the soil, but each of the others produced a shoot, which showed infection as soon as it appeared above ground. The remaining seven of the fourteen staked were left in the patch and kept under observation. On May 10, when they were 3 to 6 inches high, all were found to be infected with *Plasmopara halstedii*, except one plant, which was entirely free from infection, as were many others in the immediate vicinity. Two of these plants were now dug up, and portions of the stems at their junction with the rhizomes were fixed in various strengths of Flemming's killing fluid. Paraffin sections cut from this material and stained showed abundant mycelium in all parts of the stem except the fibrovascular bundles, the mycelium being entirely intercellular with globular haustoria extending into the cells, as shown in figure 1. The presence of the mycelium in the stem at its junction with the rhizome shows that the infection was systemic and probably came from the rhizome in the beginning.

[1] The writer searched many times in the tissues of all stages of maturity for resting spores, but without success.

The remaining five of these seven infected plants were carefully dug up, the stems cut off at their junction with the rhizomes, washed very clean with a brush, and disinfected in corrosive sublimate for five minutes. After this they were planted in steam-sterilized soil in the greenhouse, in which there had never been any *Plasmopara halstedii*. On May 23 two shoots broke through the ground; and three days later, when one was 1 inch and the other 2 inches high, they were covered with jelly glasses in order to keep the atmosphere moist. On this date the initial leaves appeared chlorotic, but no spores of *Plasmopara halstedii* could be found. The next day the lower surfaces of the leaves were almost covered with a glistening white coat of conidiophores and spores, which on microscopic examination were found to be the conidia of *Plasmopara halstedii*. Of the three remaining rhizomes, two failed to come up, while the third sent up a spindly shoot on June 5. This shoot was treated in the manner already described and the fungus fruited in the same way.

This experiment showed that the diseased plants grown in the greenhouse manifested the same symptoms as those grown in the open. It also showed that the mycelium of *Plasmopara halstedii* may be present in the rhizome of *Helianthus diversicatus*, and this, coupled with the observations described, strongly suggests that *Plasmopara halstedii* is perennial in the rhizomes of *Helianthus diversicatus*.

CONCLUSIONS

As seen from these investigations, several species of the Peronosporaceae live over from one growing season to another by at least two means: Resting spores and perennial mycelium. As is well known from the excellent studies of De Bary (2), the oospores germinate after a rest period either by zoospores or germ tubes and cause the infection of plant tissues. Because of their extremely ephemeral nature, the conidia hardly merit consideration as resting organs, but, nevertheless, they may under certain conditions function as such. If a fungus has two or more annual host plants, it may spread to one or more by conidia after primary infection has resulted from oospores on one; or the fungus may be perennial in one host and spread to another by conidia borne on the former—e. g., *Phytophthora infestans* on the potato and tomato.

The species of Peronosporaceae known to have perennial mycelium are given in Table II.

TABLE II.—*Species of Peronosporaceae having perennial mycelium*

Name of fungus.	Name of host.	Authority.
Phytophthora infestans	*Solanum tuberosum*...	De Bary (1), 1861, Bonn, Germany.
Do	.....do.....	Jensen (6), 1887, Nerilly, France.
Do	.....do.....	Melhus (12), 1913, Houlton, Me.
Phytophthora cactorum	*Panax quinquefolium*.	Rosenbaum (14), 1914, Ithaca, N. Y.
Cystopus candidus....	*Capsella bursa pastoris*.	Melhus (12), 1913, Madison, Wis.
Do	*Lepidium virginicum*..	Do.
Plasmopara viticola...	*Vitis vinifera*.....	Istvanffi (5), 1904, Budapest, Austria.
Plasmopara pygmaea..	*Hepatica acutiloba*....	Stewart (15), 1910, Ithaca, N. Y.
Plasmopara halstedii..	*Helianthus diversicatus*.	Melhus (12), 1913, Madison, Wis.
Peronospora dipsaci...	*Dipsacus fullonum*.....	Kühn (8), 1875, Halle, Germany.
Peronospora schachtii..	*Beta vulgaris*.....	Kühn (7), 1872, Halle, Germany.
Peronospora alsinearum.	*Stellaria media*.....	Magnus (9), 1888, Berlin, Germany.
Peronospora grisea.....	*Veronica hederaefolia*..	Do.
Peronospora effusa....	*Spinacia oleracea*.....	Do.
Do	*Atriplex hortensis*.....	Do.
Peronospora ficariae...	*Ranunculus ficaria*....	De Bary (3), 1876, Bonn, Germany.
Do	*Ranunculus fascicularis.*	Melhus (12), 1913, Madison, Wis.
Peronospora parasitica	*Lepidium virginicum*..	Do.
Peronospora viciae....	*Vicia sepium*.....	Melhus (13), 1915, District of Columbia.
Peronospora rumicis..	*Rumax acetosa*.....	De Bary (3), 1876, Bonn, Germany.

There can be no doubt that the mycelium of several species of Peronosporaceae may become perennial. Of course this can take place only when the host is a winter annual, biennial, or perennial, and quite generally infected. Such plants may live through the winter and renew activity in the spring, when the fungus may sporulate and spread the disease.

The perennial nature of the mycelium of other species of the genus Phytophthora has not been studied critically, but there is reason to believe that *Phytophthora infestans* is not the only one that may become perennial. In many cases other species produce oospores prolifically. Butler and Kulkarni (4) believe that on Colocasiae *Phytophthora colocasiae* may survive the dry seasons of India in the mycelial stage. Another case of perennial mycelium is that of *Phytophthora cactorum* on ginseng (*Panax quinquefolium*), a perennial having a fleshy root, described by Rosenbaum (14). The Phytophthora fungus flourishes on the roots, and, according to this author (14), can spread from the roots up the stem to the surface of the soil, and produce conidia which infect the foliage, a case very analogous to *Phytophthora infestans*.

Table II shows that, so far as known, only one species of Cystopus has perennial mycelium—that is, *Cystopus candidus* on two hosts, *Lepidium virginicum* and *Capsella bursa pastoris*. Both of these plants may be either annuals or winter annuals, and in both the fungus may become systemic and may survive the winter, provided the host plants live. Unlike *Phytophthora infestans*, *Cystopus candidus* produces oospores pro-

fusely in these two host plants after they mature or are killed by the parasite, but the writer has been unable to find oospores in the young plants during the fall, and this agrees with Magnus's (9) report that oospores are not produced in the seedling plants of spinach infected with *Peronospora effusa* in the fall. Magnus also states that the same is true in the case of *Stellaria media* and *Veronica hederaefolia* infected with *Peronospora alsinearum* and *Peronospora grisea*, respectively.

The number of species of the genus Peronospora that may survive the winter in the mycelial stage are more numerous. Table II shows nine. Careful study is in progress in regard to the remaining species of this genus. As also shown in this table, there are three species of Plasmopara which may survive the winter in this stage, and this number, the writer is confident, will be increased by further studies.

SUMMARY

(1) There are at least several species of Peronosporaceae belonging to four genera that may be perennial in the tissues of their hosts, the mycelium passing the winter either in the aerial or the underground organs of winter annuals, biennials, or perennials.

(2) *Phytophthora infestans* is not an exception in the family to which it belongs as regards perennial mycelium.

(3) The rôle of the mycelium of *Phytophthora infestans* in the tubers of its host is not an unusual one. It may grow from the tubers up the stem to the surface of the soil, sporulate, cause foliage infection, and bring about an epidemic of the disease.

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PLATE III

Fig. 1.—*Cystopus candidus* on *Lepidium virginicum*. This plant was chopped out of the frozen ground on February 22, 1911, and brought into the greenhouse. There days later white pustules of *Cystopus candidus* began to appear on the leaves.

Fig. 2.—*A*, The two leaves at the left show the amount of sporulation of *Peronospora parasitica* on leaves of *Lepidium virginicum; B*, the two leaves at the right show *Cystopus candidus* fruiting on leaves of *Capsella bursa pastoris*. The pustules developed from mycelium alive in the plants in the winter of 1911.

Fig. 3.—*Peronospora viciae* on *Vicia sepium*. A systematic infection of the downy mildew collected on April 15, 1914, in the District of Columbia. This plant was badly infected when coming through the ground.

1
A
B
2
3

HIBERNATION OF PHYTOPHTHORA INFESTANS IN THE IRISH POTATO

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INTRODUCTION

How *Phytophthora infestans* perpetuates itself from year to year has been studied ever since Unger in 1847 (34) [1] finally proved that the fungus causing the disease is a species of Peronospora. No sooner had this fact been established than students began searching for resting organs like those so common in other species of Peronosporaceae. As is well known, progress was slow, and the question as to whether *P. infestans* does or does not have oospores ended in a controversy between W. G. Smith (30) and De Bary (4) in the early seventies of the last century. The outcome is too well known to need repetition; suffice it to say that De Bary's negative evidence has been generally accepted.

Recently the oospore question has been taken up anew and bodies resembling oospores have been found by Jones (15, 16, 17), Clinton (9), and Pethybridge and Murphy (27) in pure cultures of the fungus. Although no direct claims that similar bodies exist in the potato plant (*Solanum tuberosum*) have been made, these recent investigations have at least weakened the perennial-mycelium theory, which probably was first advanced by Berkeley in 1846 (5). Like many of the botanists during the first half of the last century, Berkeley unfortunately submitted no experimental evidence to support his contention. The credit of first submitting such evidence belongs to De Bary, who in 1861 in an interesting paper (1) showed that the conidia can not live over winter; that no relation exists between the mycelium of *P. infestans* and of the saprophytes that occur on diseased tubers; that it is impossible to infect potatoes with any of the Peronosporaceae that occur on plants common about potato fields; and that the potato fungus is able to spread from diseased seed tubers up into the shoots, sporulate, and renew infection on the foliage.

About 10 years later, Scholtz, Bretschneider, Peters, and Reess took up for the "Central Commission für das Agrikulturchemische Versuchswesen" the problem how *P. infestans* perpetuates itself. They were unable to confirm De Bary (1), and Pringsheim (29), who sum-

[1] Reference is made by number to "Literature cited," p. 100–102.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 2
Oct. 11, 1915
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marized their work, offered the alternate-host theory as a final resting place for this unsolved problem.

The fact that none of these investigators was able to confirm De Bary (1) and the announcement of W. G. Smith in 1875 (30) that he had found the oospores of *P. infestans* doubtless influenced the Royal Agricultural Society to ask De Bary to again take up a study of how this fungus perpetuates itself. In a report to the Society in 1876 De Bary (4) makes the following general statement (p. 265), based on his observations and experiments, which shows plainly his thorough understanding of the habits of *P. infestans*.

> I was, perhaps, the first to call attention distinctly to the fact that the mycelium of *Phytophthora*, like that of parasites living in many other perennial plants, can be perennial in the surviving parts of the hosts, i. e. in the case of the potato, in its tubers.

It has already been pointed out that Berkeley (5) first suggested that the mycelium of *P. infestans* is perennial in the potato tuber. Many attempts have since been made by Jensen (14), Boehm (6), Smorawski (32), Hecke (12), and others to duplicate De Bary's (4) experiments both in the laboratory and in the field, but no one except Jensen has obtained confirmatory evidence, and his evidence has failed to strengthen the perennial-mycelium theory.

Naturally the accumulated negative evidence has led many to doubt the perennial capacity of the mycelium and to substitute widely different hypotheses. At least six theories as to the yearly advent of this disease have been advanced at various times: (1) That the mycelium lives over winter in the soil; (2) that mycelium is perennial in the diseased tuber; (3) that resting spores are produced which function in renewing infection; (4) that the mycelium is latent in the potato plant; (5) that the fungus fruits on the parent tuber in the soil and the spores reach the surface and cause infection of the foliage; and (6) that sclerotia-like bodies or a mucoplasm gives rise to infection. The second of these is the only one supported by any amount of experimental data, the other five being based chiefly on negative evidence, of which there is considerable.

In this paper are recorded data obtained in the laboratory and field supporting the perennial-mycelium theory.

EXPERIMENTAL STUDIES

The present study has to do largely with the function of the mycelium of *P. infestans* in infected tubers and its relation to the progeny of the host plant. The spread of the mycelium in tubers and sprouts was considered first and followed by further experiments to determine the relation of the mycelium to the shoots and young plants. Later, infected tubers were planted in the field and the progeny watched for any evidence of the disease.

RELATION OF MOISTURE AND TEMPERATURE TO THE SPREAD OF THE MYCELIUM OF PHYTOPHTHORA INFESTANS IN THE TUBERS

In order to learn something as to the influence of environmental factors upon the spread of the mycelium, tubers naturally infected with *P. infestans* were taken and the boundary line of the infected area marked with india ink. Thirty-three tubers were thus treated and buried in steam-sterilized sand in boxes 6 inches deep. The sand was kept continuously well moistened. The infected areas included from 20 to 90 per cent of the total surface area of the tubers, but there were at least two living eyes. The boxes with these tubers were kept in a greenhouse where the temperature was held at 16° C. at night and 22° in the daytime. After 12 days the tubers were all taken up and the boundary line between the sound and infected areas again traced. In every case the fungus was found to have made progress at some point on the tuber, but the progress was not uniform, the lines coinciding at some points and diverging as much as 1 inch at others. The spread seemed to have been more rapid in the vicinity of the eyes, although this was not always the case. On 7 of the 33 tubers sprouts were found that were infected with *P. infestans*, which at the time of planting, 12 days earlier, were sound.

Again the tubers were planted in the moist sand and allowed to develop for 9 days more. When dug up this time, the fungus was found to have spread over all the remaining sound surface area, except in the case of two tubers, and even in these it had made material growth. This time four more sprouts were found to be infected with *P. infestans*, its presence being proved by cutting the sprouts off and holding them for 24 hours in a moist atmosphere, the fungus in the meantime fruiting on them. Under the conditions of this experiment, therefore, it was found that in three weeks *P. infestans* had spread over 10 to 80 per cent of the surface area of the tuber, that in 11 cases it had spread into the eyes and traveled out into the sprouts, and that in the majority of the cases it spread most rapidly in the vicinity of the eyes.

This experiment was repeated under the same conditions, except that the sand was kept very dry and the tubers were held in it for six weeks. Without stating any of the details, which were much like those already given, it may be said that the fungus spread very slowly; and, while there was growth in some cases, in many the infected area remained as it was in the beginning. The tubers remained free from soft rots and germinated freely. From the results of this experiment it is very strikingly evident that to produce rapid spread of the mycelium in the tubers the sand must be kept well moistened.

In still another experiment the temperature was reduced instead of the moisture, the former being held at 4° to 6° C. In this case the fungus made little or no growth or spread in the tubers, and the potato gave as little evidence of activity, showing that both moisture and temperature exert a marked influence on the growth of the mycelium.

SPREAD OF THE MYCELIUM INTO THE SPROUTS

When it had been shown that the mycelium was alive in the tuber, at least at some point, its spread into the sprouts was studied. Three boxes (18 by 18 by 6 inches) were filled half full of soil which had never grown a crop of potatoes and which had been steamed for 40 minutes in an autoclave at 15 pounds' pressure. Twelve tubers were partially buried in each box, four of which were sound, the remaining eight being infected with *P. infestans* when harvested. The soil was well moistened with distilled water, and each box covered with a pane of glass. Each box in the series was held at a different temperature—that is, 15° to 20°; 20° to 22°; and 23° to 27° C.

The 8 infected tubers subjected to a temperature of 15° to 20° produced many sprouts, 5 of which became infected during the period under observation. The tubers subjected to 20° to 22° also produced 5 infected sprouts, these appearing during the first 14 days after planting. The greatest number of infections were obtained from the 8 diseased tubers held at 23° to 27°, 13 sprouts becoming infected during the first 14 days after planting. The checks remained free from infection. *P. infestans* seldom sporulated on the parent tuber unless the corky layer was broken, but it was very common on the basal portion of the sprouts growing from infected tubers. In many cases the eyes producing infected sprouts were cut out to learn whether the fungus was present in the tissues immediately surrounding them, and in every case it was found. This showed that the sprout infection was due to the spread of the mycelium and not to spores present in the air, for had the infection been due to spores the checks would have shown as high a percentage of infection as the diseased tubers. Infection by *P. infestans* occurred on sprouts of all sizes, from those barely visible to those nearly 1 inch in length. It was a very common occurrence to find the fungus sporulating first on the lower third of the sprouts, while on the upper two-thirds it was not apparent, but it required only one or two days for the remaining portion to become covered also, which indicates the rate of spread of the mycelium in the sprout tissue.

Naturally discoloration and decay followed the fructification of the fungus. Plate IV, figure 2, shows a potato with diseased and healthy sprouts. This is a late stage of sprout infection, and the tissues of the two infected shoots have blackened. The healthy sprout stands on a portion of the tuber showing no external evidence of the disease, while that part surrounding the diseased sprouts is infected with *P. infestans*. The fungus sporulated only on the sprouts of the diseased tubers, while those arising from the healthy tubers in the same box remained sound throughout, which makes it certain that infection was not by spores present in the air or soil, but by the migration of the mycelium in the tissues of the parent tuber.

This experiment was repeated and has been reported in full in an earlier paper (21). Except in one particular, the results were, in general, alike. In this case a sprout grew out near the surface of the soil from one of the infected tubers. This sprout became infected and the mycelium of *P. infestans* grew out from it into the soil for a distance of about 1 cm. This is not a usual occurrence and happens only when conditions are very favorable for the growth of the fungus. A slight decrease in the moisture content of the soil and the fungus is no longer in evidence, nor does it return if the original moisture condition is restored.

This experiment was again repeated on January 29, but only two sets of temperatures, 15° to 20° and 23° to 27° C., were used. The other set of temperatures was omitted because the supply of tubers was rapidly becoming exhausted, and, besides, it had been shown that temperatures between 15° and 27° were the most favorable. The results were, in general, like those already recorded and need no further consideration. From this series of three experiments, in which infected tubers were partially buried in moist, sterile soil, it is clearly shown that the mycelium of *P. infestans* in infected tubers spreads from the parent tuber into the sprouts, where it may sporulate freely.

Naturally the next step was to learn something as to the behavior of the infected tubers when wholly buried in the soil. To this end 12 sound tubers of the Irish Cobbler variety were artificially infected with a zoospore suspension held in contact with a sprout about one-fourth of an inch long by means of a ring of paraffin, as shown in Plate IV, figure 2. These tubers, together with 6 sound ones as controls, were buried 2 inches deep in a box of wet sterilized soil and placed in a saturated atmosphere at 23° to 27° C. The tubers had gone through the rest period, and in some cases the sprouts were 1 inch long. Eleven days after planting, 4 of the tubers had thrown up shoots. The remaining 8 were dug up to learn their condition, and it was found that in every case the fungus had spread into sprouts other than the one originally infected. Plate IV, figure 2, shows a tuber with the paraffin about the infected eye and the cluster of 5 sprouts at the bud end of the potato. One of the cluster, it should be noted, is free from infection. After the tuber was photographed it was cut and the discoloration typical of *P. infestans* was found at the base of the sprouts. That it was *P. infestans* was further shown by the production of spores and conidiophores on the discolored tissue. The fungus had spread from the initial point of infection over to the point where the cluster of infected sprouts originated from the parent tuber. The four shoots that came through the ground were allowed to remain until April 30, when they were dug up. These were found to be sound, while the parent tubers were totally decayed. The controls remained free from infection by *P. infestans* throughout and developed into normal plants.

In this experiment 4 of the tubers produced healthy plants, while the 8 others were completely overrun before any of the sprouts could reach the surface of the soil. This explains why seed potatoes infected with *P. infestans* give a poor stand. It also shows that the relation of the fungus to the sprouts is the same whether the tubers are wholly or only partially buried. Another significant fact brought out in this experiment was the presence of spores on the surface of the infected sprouts in the soil. This was especially true on sprouts attacked but not wholly killed.

GROWTH OF THE MYCELIUM UP INTO THE SHOOTS

When it became evident that the fungus could grow out into the sprouts from an infected tuber partially or wholly buried in the soil, experiments were outlined to ascertain whether it might not also grow up into the shoots. Thirty tubers were artificially inoculated by introducing living mycelium from pure cultures of *P. infestans* into a wound in each, and all were immediately planted in pots in the greenhouse, the same number of healthy tubers being planted as checks on the same date. None of the plants growing from these tubers showed any signs of infection with *P. infestans*, although they were watched carefully for 71 days, after which the experiment was terminated.

In another experiment 12 naturally infected tubers were planted in pots of steam-sterilized soil. The same number of healthy tubers were planted at the same time as checks. Only 4 of the 12 infected tubers came up, and 3 of these were much less vigorous than the controls. The spindly, sickly looking shoots that grew from the diseased tubers were watched for 47 days, but no sign of *P. infestans* was noted. The tubers were then dug up and found to be wholly decayed, but the stems were sound.

In a later experiment 200 naturally infected tubers were divided into four equal lots and planted directly on the greenhouse bench 1, 2, 3, and 4 inches deep, instead of in pots. An equal number of sound tubers were planted in a like manner as checks. Conditions were made highly favorable for the growth and development of the plants. Seven days after the tubers were planted, a few shoots were noted coming through the ground. The following germination was obtained (Table I).

TABLE I.—*Percentage of germination of potato tubers infected with Phytophthora infestans*

Number of days after planting.	Percentage of germination of seed planted—				Percentage of germination of checks.
	1 inch deep.	2 inches deep.	3 inches deep.	4 inches deep.	
26	33	39	33	13	96
38	39	39	45	44	98

Of the 78 plants that came up 21 were markedly abnormal, while the remaining 57 were quite sound. The sickly plants were covered with bell jars for several days at a time so as to make the moisture conditions more favorable for *P. infestans*, but not a single case of infection either on the basal portions of the stems or on the foliage was found, although the plants were examined daily until the vines died down.

From these experiments and others of a similar nature not mentioned here, it is plain that environmental conditions and the stage of development of the tuber planted determine whether the mycelium may or may not grow up into the shoots. The conditions prevailing in the ordinary greenhouse are not suited to the spread of the mycelium up into the stems.

Believing temperature and moisture to be the chief environmental conditions bearing on the development of *P. infestans*, experiments were made to determine the influence of these factors on the disease.

Temperature.—The influence of temperature was considered first. Three experiments were made, and, as all were practically the same, a description of one will suffice.

Five 12-inch pots were nearly filled with soil and steam-sterilized. On January 29, 1912, three tubers infected with *P. infestans* were planted 2 inches deep in each of three of these pots, and in the two remaining pots sound potatoes were planted as controls. Two of the pots were placed in a greenhouse where the temperature varied from 15° to 20° C., depending upon the time of the day; the third was placed in another greenhouse where the temperature ranged from 23° to 27° C. With each was placed a pot containing healthy tubers.

The first shoot to appear in the pots kept at 15° to 20° C. came up on February 6, or 8 days after the tubers were planted. The healthy tubers used as controls did not come up as soon as the diseased ones. They were more dormant at the time of planting. It has been observed by several investigators that tubers infected with *P. infestans* germinated sooner than healthy ones. In 12 days all of the diseased tubers had shoots up so high that the panes of glass covering the pots had to be removed. In order to keep the young potato plants in a moist atmosphere, a large bell jar was placed over each of the three pots. Careful examination was made daily. On March 18, or 45 days after planting, the plants were 7 inches tall, but showed no signs of *P. infestans*. At this time the plants held at 15° to 20° C. were dug up to learn the condition of the diseased tubers planted. Three were wholly decayed, while the other three were only half rotten and showed on the remaining portion the typical shrunken areas so characteristic of this fungus. All of the tubers in the control pot were sound. The three tubers partially decayed were now placed in a moist chamber in order to ascertain whether the fungus was still alive in them after being buried 45 days

and after having nourished several plants to partial maturity. Two days later an examination showed that spores and conidiophores were developing on two of the tubers; but no indication of infection was observed on either the leaves or stems which were placed in a moist chamber. Examination on the following day showed no further developments, and, as the potato plants were becoming very much discolored, the observations were discontinued. It should be noted at this point that the fungus was alive and able to sporulate on the diseased tubers after being in the soil for 45 days at a temperature between 15° and 20° C. Had the fungus been latent in the potato leaves and stems, as claimed by Massee (20), it should have developed. The most interesting and important fact brought out in this experiment was the production of healthy vines by a tuber having in it the mycelium of *P. infestans* which remained alive for 45 days.

The two pots which were kept at 23° to 27° C., one containing three infected tubers and the other three healthy tubers, came up a little earlier than those kept at 15° to 20° C. The first shoot came up on February 4, or 6 days after planting, and in 10 days all three of the diseased tubers had shoots up, some of them longer than others. The development of the tubers used as controls was several days behind that of the diseased tubers. Ten days after planting, the shoots were so tall in the pot containing diseased tubers that the pane of glass had to be replaced by a bell jar. The control was treated similarly. Nothing of special interest occurred until March 8, or 39 days after the tubers had been planted, when it was noticed that one of the small shoots growing from one of the diseased tubers appeared water-logged at and a short distance above the surface of the soil. It did not have the normal appearance common to the stems of the other seven shoots in the pot. Upon examination of the water-logged area with a hand lens, a white glistening growth could be seen on the surface. Some of this material was carefully removed and examined microscopically and proved to be spores and conidiophores of *P. infestans*. This infected plant was about 2 inches tall, spindly, light green, and less robust in appearance than some of the other plants in the same pot (Pl. V). The soil was carefully dug away from the stem, and a portion of it below the soil was found to be diseased. This portion gradually became darker as it approached the mother tuber, being brown and doubtless dead at the point of attachment. The parent tuber was nearly all decayed, except one small portion, which was still firm and from which the diseased shoot in question had developed. Free-hand sections made of the portion of the parent tuber where the stem was attached showed the presence of a nonseptate fungous mycelium which was undoubtedly that of *P. infestans*. The tissues of the stem nearest the mother tuber were softer than those higher up, which would indicate that the infection was of longer standing in that section of the stem.

The controls remained free from infection. Because of possible contamination, no further observations were made in the remaining plants in this pot.

This experiment was repeated, beginning February 22, 1912, but instead of large pots six boxes 18 by 18 by 6 inches were employed. Diseased tubers were planted in four of these and sound tubers in the remaining two. Eight were planted in each box, the conditions being exactly the same as in the preceding experiment.

On March 3, or 11 days after planting, one shoot was found just breaking through the soil in one of the two boxes at 23° to 27° C. It seemed perfectly normal both in color and in size, but on examination the next day both the shoot and the surface of the soil immediately surrounding it were covered with a white glistening fungous growth resembling that of *P. infestans*. Upon examining this growth microscopically it was found to be the potato fungus, as suspected. The mycelium on the soil had grown out from the infected shoot and seemed to be confined to the surface of the soil. The soil about the shoot was removed and the underground portion of the stem exposed. It was found to be water-logged just below the surface of the soil and was gradually becoming brownish as the parent tuber was approached. An examination of the parent tuber showed it to be badly decayed at one end, but quite firm at the other. The tissue of the tuber was examined at the base of the young shoot and showed the characteristic blackening due to *P. infestans*. After 48 hours in a moist chamber the fungus fruited profusely. Plate IV, figure 3, shows a cross section of the tuber and the infected shoot.

Moisture.—As stated earlier, moisture influences in some way the behavior of the seed tuber and the fungous mycelium contained therein. It was thought worth while to hold infected tubers in comparatively dry rather than very moist soil, as was done in the preceding tests. To this end 24 infected tubers with several living eyes each were planted in steam-sterilized soil on January 13, 1914, in a house where the temperature varied from 15° to 20° C. After 30 days they were covered with a glass-house and kept well watered. Ten of the tubers rotted in the ground before producing any shoots. Thirteen days later a small, spindly shoot growing from one of the tubers showed discoloration just at the surface of the soil. This infection spread upward and the fungus fruited the following day. The remaining 13 were allowed to stand two weeks more, but none of them became infected. When dug up, it was found that all the mother tubers were rotten except two. In these *P. infestans* fruited when the tubers were cut open and laid in a moist chamber, showing plainly that the fungus may remain alive in the parent tubers for at least two months under the conditions of this experiment and also that the mycelium may spread up the stem, even though the infected tuber is not held continuously in wet soil.

In order to test still further the effect of moisture on the growth of the fungus up into shoots, 12 vigorously germinating tubers of the Green Mountain variety were planted in only slightly moist, steam-sterilized sand. These tubers grew rapidly, and in six days some of the sprouts began to break through the surface of the sand. Twelve days later 2 of the 12 tubers were dead. The remaining 10 were potted in steam-sterilized soil and placed in a glasshouse where the soil was well watered and the humidity high. Nine days later one shoot of one of the tubers was badly discolored near the surface of the soil. The discoloration spread up the stem, and after two days the infected area bore conidiophores of *P. infestans* in considerable abundance. When the tuber was dug up, the shoot was found to be diseased throughout its whole length below the surface of the soil. Six days later another tuber showed an infected shoot like the one just described. The remaining 8 mother tubers were dug up two weeks later and found to be entirely decayed. These results tend to show that continuous high moisture content of the soil is not necessary for the growth of the mycelium in the tuber up into the stems. According to the results obtained in these experiments, the soil may be kept comparatively dry until the plants are up. Furthermore, under these conditions the tubers do not rot as rapidly, and a larger number of shoots are produced by each.

INFECTED SEED POTATOES THE CAUSE OF AN EPIDEMIC OF PHYTOPHTHORA INFESTANS

The relation between seed potatoes infected with *P. infestans* and the development of epidemics of the disease under field conditions has received consideration both in Europe and in America, but no one has yet been able to trace and establish beyond doubt the existing relationship. Both De Bary (1, 4) and Jensen (14) claim to have done so, but they made only limited tests in the open in gardens, where conditions are not always comparable to those existing in the field. A large number of field trials having been made with only negative results, coupled with the fact that the mycelium grew up into the stems under laboratory conditions, led the writer to make field trials. For this purpose a section of the country was chosen where this disease occurs annually—namely, northern Maine. Such a section should afford the environmental conditions suitable for the development of all phases of the disease.

FIELD STUDIES IN 1913

The land selected for the experiment had not grown a crop of potatoes for at least five years and had been in hay for the preceding four years. The infected seed planted was selected in the spring from five bins (1,200 bushels each) of potatoes, Irish Cobbler and Green Mountain varieties, grown in the vicinity of Houlton, Me., and held in storage

through the winter. The tubers selected showed various stages of infection; but none were used that did not show at least one living eye (bud). On June 6 the tubers were planted in a 2-acre field of potatoes somewhat isolated from adjoining fields, 256 being planted whole in two rows 8 rods long. In a third row 162 hills were planted with cut infected seed. Alternating with these, three rows were planted with healthy seed, Green Mountain variety, as checks. The seed was planted between 1 and 2 inches deep and the row hilled up so as to cover the sets from 3 to 5 inches. Continuous records were taken of the soil temperature by means of a self-registering Richard soil thermograph. A record was also kept of the rainfall, especially as to the date and approximate amount.

As would naturally be expected, the infected whole tubers sent up shoots more rapidly than the cut seed. Six of the whole tubers had shoots through the ground two weeks after they were planted. On July 6, 30 days after planting, 63 per cent of the whole infected tubers had shoots up; so also did 49 per cent of the cut infected seed and 97 per cent of the tubers planted in the three control rows. After July 6 the percentage increased very little in any of the foregoing cases. On this same date six of the whole diseased tubers that had failed to send up shoots were dug up for examination. Four of these were dead and nearly decayed, while the remaining two had two and five shoots, respectively, which were just ready to break through the surface of the soil. Plate VI, figure 2, shows the condition of one of these shoots immediately after digging. They were taken to the laboratory later and examined for spores of *P. infestans*, but none were found. Subsequently they were placed in a moist chamber overnight, and the next morning small patches of conidiophores bearing spores, which on microscopic examination proved to be those of *P. infestans*, were found scattered over the diseased areas. The infected shoots were very much like those obtained in the laboratory experiments discussed earlier. It should be noted that a few days before the plants were dug up a light shower of rain had fallen, which, it is believed, materially aided the progress of the fungus. These developments in the field experiments are wholly comparable with those in the laboratory, in which the sprouts were attacked and overrun by the disease before reaching the surface of the soil.

On July 13 a very interesting case developed in the row planted with infected cut seed. When the infection was first noted, the discoloration had extended up the stem of the plant only half an inch above the surface of the soil. There was no evidence of spores of *P. infestans*. The weather was clear and the humidity unusually low, a condition not favorable for sporulation of *P. infestans*. The plant was carefully watched the following day, but no evidence of sporulation could be detected. The next

morning, however, the fungus, which, on microscopic examination proved to be *P. infestans*, had fruited, a 500 c. c. beaker having been inverted over the plant in the evening. For three successive mornings after this date evidence of a new crop of spores of this fungus on the little plantlet was found (Pl. VII, figs. 2 and 3). Later the plantlet fell over, owing to destruction of tissue by the fungus and soft-rot organisms which followed. The stem was found to be discolored all the way down to the parent tuber, a distance of 6 inches. The plant was allowed to remain in the field in order to ascertain whether it might infect the foliage of surrounding plants, but no infection developed and the plantlet soon died and dried up. Conditions were probably unfavorable in this case for the development of secondary infections, owing to a poor stand in the row where this infected plant happened to be. This condition makes it necessary for the spores to be carried a greater distance than might have been the case had a higher percentage of the seed planted in this row grown. The stand in the row in question and also the infected hill are shown in Plate VII, figure 3, This case is of special interest in showing that no further development of the fungus occurred, although it did grow up the stem from the diseased parent tuber to the surface of the soil and sporulate.

It was not until July 25 that another case of infection by *P. infestans* was discovered on any of the six rows under experimentation. This case developed in one of the hills growing from a whole diseased tuber. The hill was a vigorous one with 13 shoots, all normal except 3. The smallest of these 3 was 6 inches tall, while the others were fully twice this height. The plantlet was well shaded by the others and was detected only on careful examination of the hill (Pl. VII, fig. 1). When first found on July 25, fully an inch of the stem above the surface of the soil was discolored and a hand-lens examination showed that a fungous growth was present. Some of this growth, collected on a slide and examined microscopically, proved to be spores of *P. infestans*. The weather for five or six days previous to July 25 had been rainy, cool at night, and quite warm in the day time, conditions highly favorable for the rapid growth and spread of the fungus, as demonstrated in the laboratory studies.

The infection spread up the stem into the petioles of the lower leaves and produced spores in abundance. On the 29th, or four days after the infection was first noted, two leaflets in the hill showed infection, and discolored areas appeared on the stems of three of the adjoining shoots about 2 inches above the surface of the soil. The next morning five new leaflets in the hill showed early stages of infection. These infections occurred on leaves in the lower third of the hill, and each day the number of infections increased on the foliage. On July 31 one leaflet was found infected near the top of a plant in one of the adjoining

check rows, and as there was no other evidence of infection in this entire row it seemed quite certain that the spores had come from the hill previously mentioned. On August 5, six days after this stray infection was first noted, 14 others were found immediately below it on the leaflets in the same hill. It seemed quite apparent that the spores had fallen from the infection above and infected the leaves below. The disease continued to spread rapidly until August 10, when a period of hot, dry weather for 10 days checked its development temporarily. At the end of this dry spell, however, it resumed activity, and an epidemic of blight was well under way in this portion of the field. All the plants in the plot, except those on a few short rows of a foreign resistant variety, were killed by late-blight before frost. Four other cases, similar to the one just described, developed between July 25 and August 4. The symptoms in all cases were the same and need not be repeated. In each case the spores produced by the initially diseased shoots infected adjoining foliage and became centers for the spread of the disease.

The plants in the three alternating rows planted with healthy seed were watched for evidence of stem and foliage infection as carefully as those planted with infected seed, as was also the rest of the 2-acre field, but in no case did any infections develop that could not be traced to the centers in the rows planted with infected seed. Of course, after the epidemic was well under way, the source of any single infection was unknown. The significant point and the one on which information was desired was the origin of the very early stages in the development of an epidemic and not the late.

The results of the field tests of 1913 may be briefly summarized as follows: (1) Only 63 per cent of the whole infected tubers and 49 per cent of the cut infected seed grew; (2) the mycelium in infected seed tubers responded the same way in the field as it did in the laboratory experiments; (3) shoots were found that became infected before they reached the surface of the soil; (4) others infected were able to break through the soil and become centers of foliage infection. On these dwarfed infected shoots the fungus fruited and infected the foliage, first in the same hill and later in those adjoining. In this way these hills became the centers for the development of an epidemic.

FIELD STUDIES IN 1914

It is well known that too much reliance can not be placed on the results of 1-year trials under field conditions. This is especially true when dealing with a fungus like *P. infestans*, which is very much influenced by environmental conditions. In view of this fact, it seemed desirable to repeat the field trials of 1913. In 1914, a plot of ground was chosen at Caribou, about 60 miles north of Houlton, Me., where conditions are fully as favorable for the development of late-blight as at

the latter place. A plot of ground was selected that had been lying idle in 1913, but which before had grown several crops of potatoes in succession.

Tubers of the Green Mountain variety showing all stages of infection by *P. infestans* were selected on May 25 from potatoes grown and held in storage throughout the winter in potato cellars at Caribou. Most of them were badly infected, as was natural to expect at this late date. Many had only one living eye, while others, of course, had several. Both whole and cut seed were planted in the same way as already described in the field tests of 1913. In one row 170 whole tubers were planted and 363 in two rows adjoining. On each side of these three rows two rows were planted with sound seed as checks, also of the Green Mountain variety. The planting was made on June 2, when the soil was drier than usual. There was very little rain until July 20, when an inch fell, but, as a whole, the season was drier than that of 1913 and therefore was less favorable for the development of late-blight.

An examination made on July 15 showed that 47.6 per cent of the whole infected tubers, 37.4 per cent of the cut infected seed, and 92 per cent of the healthy seed in the four adjoining rows came up. The low percentage of germination of the infected seed was probably due to two factors, the large amount of infection of the seed with *P. infestans* and the dry weather following planting. The infected seed rotted in the ground in the same way as described in the studies made in 1913.

The first case of infection by this fungus was discovered on July 22, two days after a heavy rain had fallen. It was in a hill grown from a whole infected tuber having nine shoots from 12 to 18 inches tall. Five of the smaller shoots were found to be infected at and below the surface of the soil. The soil was carefully removed from about the hill, and two of the five were found to be discolored all the way from the mother tuber up to the surface of the soil. The three others seemed to have become infected at the surface of the soil, probably by spores borne on the two shoots most generally infected. The infection of neighboring stems in the same hill above the surface of the soil was also noted in the field studies of 1913.

Two days later another hill, also grown from whole seed, was found to be infected. This had 14 shoots, varying from 10 to 18 inches high. The smallest shoot was discolored in the same way as described in the previous case, and upon further investigation the infection was found to extend down to the parent tuber. The fungous infection was evident by the glistening white growth on the stem just above the surface of the soil. None of the older shoots in this hill were infected at this date.

On July 26 one of four shoots in a hill grown from cut seed was found to be infected. These four shoots ranged from 6 to 14 inches in height. Two of the smallest shoots in this hill were infected with *P. infestans*. The

hills in the four check rows were watched as carefully as those in the two rows planted with infected seed, but no infections with *P. infestans* were found.

The development of foliage infection from the three centers described was gradual and wholly comparable to that described in considerable detail in the studies of 1913. It should probably be said in this connection that the first foliage infection was found on July 27, five days after the first case was discovered. By August 14 leaves within a radius of 10 to 20 feet from each center or station were infected with *P. infestans*. A bad epidemic of late-blight was in full swing throughout the whole 2-acre field by September 10. It is plain that the three centers above described formed the starting points for this epidemic. Other centers of infection may have developed subsequently, but no attempt was made to follow the later developments because of the constant recurrence of new foliage infections resulting from the infections about the original centers. The results of the field studies of 1914 confirmed in every way the results obtained in 1913.

The fact that a tuber infected with late-blight may cause an epidemic of the disease raises the question as to the rôle of infected tubers left in the field at harvest time. The majority of these are killed by frost, but a few remain in the soil or get covered during the digging of the crop and may pass through the winter in a living condition. Observations showed plainly that many tubers survived the winter of 1913 in Aroostook County, Me. The fields planted to oats in 1914 that had been in potatoes the previous season were well sprinkled with volunteer potato plants. It is common knowledge among the growers of northern Maine that some seasons volunteer potato plants are very plentiful. Their presence or absence is determined largely by the season, especially by the time and amount of snowfall.

POSSIBILITY OF CONIDIA OF PHYTOPHTHORA INFESTANS BORNE ON THE SEED TUBER REACHING THE SURFACE AND CAUSING FOLIAGE INFECTION

In 1876 De Bary (4) called attention to the possibility of conidia on the seed tuber being able to reach the surface and cause foliage infection. Hecke (12) and Clinton (8) are inclined to believe they function more extensively than the mycelium in the seed tuber. Little is known about the production of conidia on infected potato tissue in the soil or their relation to renewing infection from one year to another. For this reason it was thought advisable to learn something about the possibility of the fungus fruiting on cut seed in the soil and whether the spores functioned.

To this end 31 infected seed pieces were planted in the usual manner on June 22, 1913, at Houlton, Me. The soil was quite dry, and the soil temperature ranged from 10° to 14° C. Three days later they were dug

up for examination, but no spores of *P. infestans* were found. They were again planted and the next day a rain fell, wetting the ground down to the seed potatoes. On June 30, four days after the second planting, the seed pieces were dug up again. Microscopic examination showed that spores and conidiophores of *P. infestans* were present on 26 of the 31 pieces and the growth of the fungus in seven cases was readily visible to the unaided eye. The spores were found to germinate freely in water. These seed pieces were again planted on July 1 and left in the ground for a period of 14 days. At this time careful examination revealed a limited number of spores on 5 of the pieces, but these spores did not appear to be normal; and when placed in water only 3 or 4 germinated. A search was also made for mycelium of *P. infestans* in the soil adhering to the seed pieces, but none was found. The plants that grew from these infected seed pieces were examined daily from the time they came up until the vines were nearly mature, but no infection by *P. infestans* appeared on the foliage.

The above experiment was repeated, beginning on July 2. In this test 14 diseased seed pieces were planted just after a light rain. Four days later they were dug up and examined; on 7 of the tubers spores of *P. infestans* were found. There was no indication that the mycelium was growing saprophytically in the soil adhering to the cut surfaces of the diseased pieces. The pieces were immediately replanted and allowed to grow throughout the season. On July 25 the stem of one of the plants showed infection at the surface of the soil. When dug up, it was found that all of the stem below the surface was diseased and also the parent tuber at the point where the stem originated. This tends to show that the mycelium grew from the parent tuber up into the young shoot and that the infection was not caused by spores in the soil. This plantlet stood in an exposed place and soon died. Spores were produced, however, and a leaf on an adjoining plant became infected. This spread slowly in the leaflet and only a few spores were produced. Finally the leaflet died and dried up and no further infections occurred on any of the plants in the same or adjoining rows. In both these experiments conidia were produced on the seed tuber, but none of them functioned in causing any infections.

In the spring of 1914 further tests were made at Caribou, Me. On June 4, 183 potato seed pieces infected with *P. infestans* were planted in accordance with common practice. The next day it rained. On June 7, 26 of the 183 seed pieces were dug up and examined for conidiophores and spores of the fungus. These were found on 9 of the pieces and the growth was abundant enough to be easily seen with a hand lens. On July 10, 12 more seed pieces were dug up and examined, but no evidence of fructification of *P. infestans* was found. The weather had been clear and warm the five preceding days and the soil was much drier than on June 7. It

may have been that spores were present somewhere on the cut surfaces, but they were not sufficiently abundant to be found even after long and careful search.

On June 20, 20 more of the 183 seed pieces were dug up and examined, but again neither conidiophores nor spores of the fungus could be found. The cut surfaces of the seed pieces in every case had either corked over or started to decay.

No mycelium could be found growing free in the soil about the diseased tubers. No evidence was obtained showing that the fungus continues to sporulate on the seed tubers in the soil. Spores are produced abundantly on the cut surfaces of tubers recently planted in moist soil only, but these disappear in the course of a week or 10 days. In an earlier part of this paper it has been shown that spores may be borne in considerable abundance on sprouts killed before they reach the surface of the soil. Whether these spores ever function in infecting other potato tissue below the surface of the soil has not been shown definitely by any of the earlier workers or by any of the writer's experiments.

There is still another possible source of conidial infection that should be mentioned in this connection. A common practice in northern Maine and other potato-growing sections is to feed the culls to hogs or to dump them in some out-of-the-way place about the farm. In the culls there are usually a considerable number of tubers infected with late-blight. When the skin is ruptured on these, the fungus may fruit. Spores borne in this way may reach potato foliage and lead to infection. Then again, as observed by the writer in numerous cases, tubers infected with late-blight are often dumped in some wet or swampy place on the farm. In two such cases an infection of late-blight was found on the mass of growing plants as early as July 25 and 29. It was impossible to determine how and where the infection originally started, but it was clear that the disease had made a good beginning. It is, of course, needless to say that if such cases developed near a potato field, it might readily become infected.

Whatever may be the possible relation of the conidia to the renewal of epidemics of *P. infestans*, two points are perfectly clear: (1) That spores are borne in the soil on the freshly cut surfaces of infected seed and on sprouts when the soil is sufficiently moist and (2) that the spores probably do not remain viable more than two to three weeks in the soil.

RATE OF SPREAD OF THE MYCELIUM OF PHYTOPHTHORA INFESTANS IN THE POTATO STEM

The rate of spread of infection in the potato stem is of interest because of its direct bearing on the growth of the mycelium from the diseased tuber up through the stem. Healthy plants from 20 to 55 cm. high were exposed to infection with *P. infestans* by spraying a spore suspen-

sion over the plants; and when infections developed on the stems their upper and lower limits were marked with india ink. The infected plants were kept in the greenhouse under conditions favorable for the normal development of the host.

Records were made of infections occurring anywhere on the stem from within 6 cm. of the ground to within a few centimeters of the top. Eight infections within 10 cm. of the ground were kept under observation for four days. The total upward spread of infection in these during the four days was 30 cm., or an average of 3¾ cm., and the downward spread was 21 cm., or an average of 2½ cm. Five infections from 10 to 20 cm. above the soil were studied. Two of these were allowed to continue for 48 hours, and the remaining three for only 24 hours. After two days the combined spread up the stem in the five cases was 11 cm., and down, 6 cm., the average spread up and down in each case being $2^1/_5$ and $1^1/_5$ cm., respectively. Three stem infections were studied that were more than 20 cm. above the soil; two were between 20 and 30 cm. and one 45 cm. After four days the total spread of infection upward was 23 cm. and downward 11 cm. The average upward growth was 7⅔ cm. and the downward 3⅔ cm.

It should be noted that in every case the spread of infection was more rapid up than down the stem and that the fungus progresses more rapidly in young than in old tissues. It is thus evident that it may require only a short time for *P. infestans* to spread sufficiently in the potato stem to reach the surface of the soil, once it is in the basal portion of the shoot. It is likewise quite probable that the fungus grows down the stem from the surface of the soil.

HISTOLOGICAL STUDIES OF THE RELATION OF THE FUNGUS TO THE POTATO STEM

The question arises as to which the mycelium uses when it grows up the infected stem, the cortex, vascular system, or central cylinder. A section of an infected stem always shows that the cortex is discolored, while the rest of the tissues are quite normal. The natural inference from this macroscopic evidence is that the mycelium used the cortex most extensively.

In order to get more exact evidence on this point, infected shoots were killed in various fixatives and were later sectioned and stained. In every case where the cortex was discolored, the cells had collapsed and took the stain very heavily, as shown in figure 1. In such cases the mycelium was not readily seen, and in the majority of cases it was absent. It was sometimes found, however, in the cells between the outer cambium layer and the inner cortical cells, but more often at this stage it was seen growing among the pith cells, as shown in figure 2. Where the cells of the cortex were more normal, or from ½ to 1 cm. above the border line between

healthy and diseased tissue, the hyphæ could be readily seen ramifying between the cells, as shown in figure 3. The mycelium can usually be found higher up in the stem in the cortex than in the pith cells when the disease is growing up the stem from the infected parent tuber. When the cortex has been destroyed it may be found in the pith cells. So far the author has seldom found the mycelium in the vascular system or the wood cells. Histological studies indicate that the mycelium of *P. infestans* spreads up the stem most rapidly in the cortical region when conditions are favorable for its rapid growth.

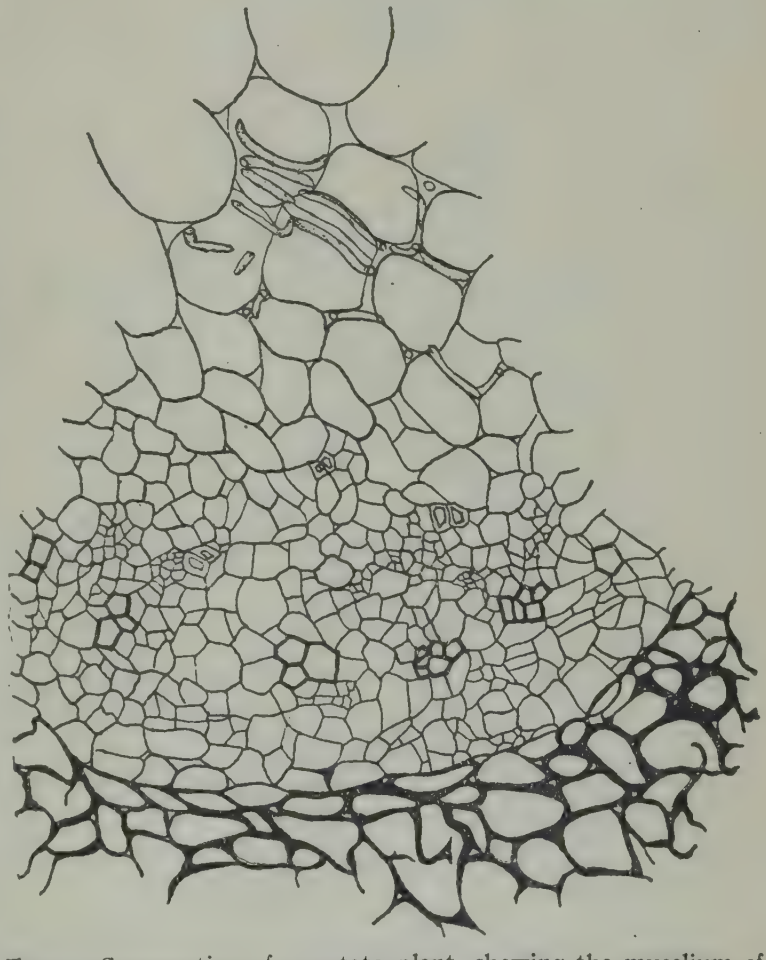

FIG. 1.—Cross section of a potato plant, showing the mycelium of *Phytophthora infestans*, which has killed the cells of the cortex and is a later stage than that shown in figure 3. The mycelium is present among the pith cells. The plant from which this cross section was made became infected like the one in figure 3.

DEVELOPMENT OF EPIDEMICS OF PHYTOPHTHORA INFESTANS

One argument used persistently against the theory of resting mycelium being the means of perpetuation of *P. infestans* is the sudden and almost simultaneous outbreak of the disease over wide areas. It has seemed more plausible to many to imagine that some form of resting spore functioned in spreading the disease rapidly each year, as is known to be the case in related species. Massee (20) has questioned the capacity of the conidia of *P. infestans* to start an epidemic. He believes that epidemics start from mycelium of the fungus latent in the tissues which becomes active with the advent of favorable weather conditions.

In the fall of 1911 the following experiment was made at Madison, Wis., to learn something as to the development of an epidemic of *P. infestans* under field conditions, with special reference to the rôle played by conidia. It should be mentioned that this fungus seldom occurs in the vicinity of Madison, and, so far as known, it was absent from the State in 1911. The writer is sure it did not occur in the vicinity of Madison that year, and therefore his results were not complicated by its presence. On the even-

ing of August 17, 1911, after a spell of wet weather, two potato plants were sprayed with a suspension of spores of *P. infestans*, the spores having been taken from infected plants in the greenhouse. The inoculation of the two plants was made in the usual way and typical spots became visible in the course of five days. The amount of infection was not extensive. The ground was very moist, owing to the fact that several rains had fallen the previous week, and the weather was continuously cloudy from August 22, the date infection first appeared, until August 27.

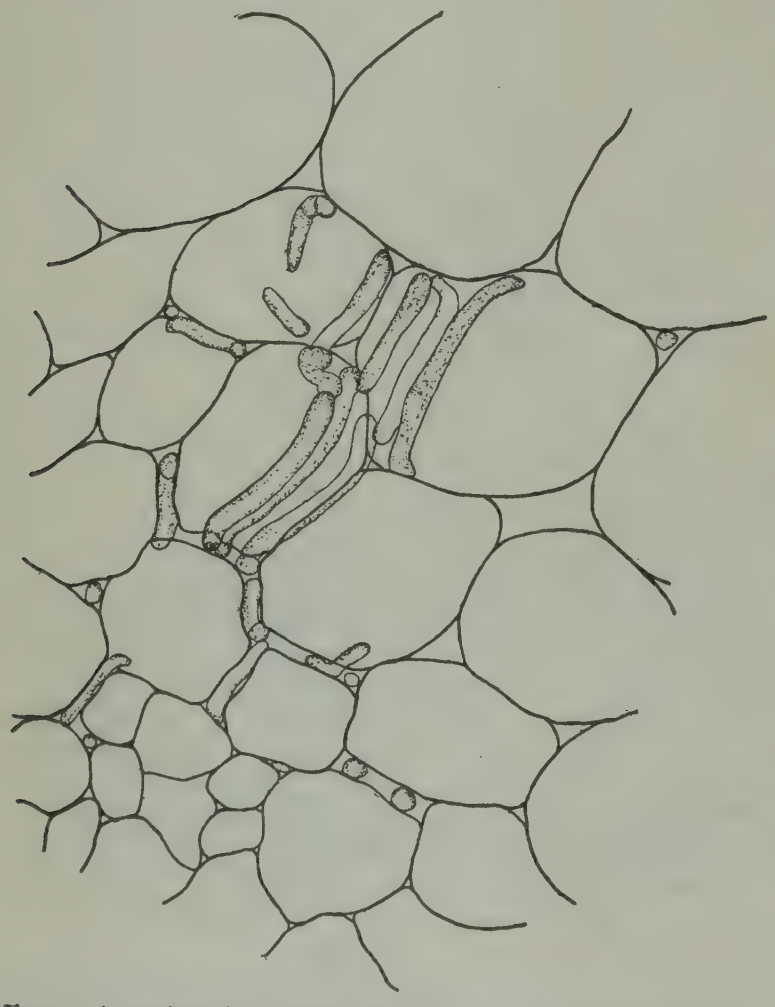

FIG. 2.—A portion of the same section of a potato plant shown in figure 1, showing the mycelium in the pith region of the stem.

On August 30 infections were found on two plants adjoining those artificially infected, and the next day four more plants immediately adjoining showed infection on several leaves. Careful examination showed no infection on any of the plants farther away. The new infections that had occurred on August 31 were on the six plants immediately surrounding the two artificially infected. The fungus had made no further spread in the half-acre potato plot. After August 30 new infections were daily found farther and farther from the two plants first infected, and on September 7 infected leaves could be found everywhere throughout the plot, though none of the vines were conspicuously blighted. By this time all the plants within a radius of 8 feet of the two plants initially infected were killed. Farther away the infection was much less in extent, though present in abundance. By September 12 the plot was very badly blighted; not a single plant anywhere was free from infection, and many were wholly dead. No further records except as to the time of harvesting and the amount of loss were kept when the tubers were harvested on October 10. Less than 50 per cent of the crop was fit to put in storage, and less than 10 per cent kept until spring, although held in good storage.

The conclusions to be drawn from this experiment are perfectly obvious. (1) An epidemic can be started by the infection of two plants in a field; (2) two infected plants can spread infection sufficiently to destroy the vines on a half-acre plot in 29 days. That a larger plot, indeed a field of many acres, could be destroyed by one infection is clearly evident.

It might be argued that these conditions were not typical of those occurring under field conditions. On October 14 a visit was made to the potato fields of western New York, where an epidemic was just starting in many of the fields. Infection centers like the one produced by artificial infection in the potato plot at Madison were in evidence in several fields. Another visit to the same fields early in November showed that they had been destroyed by an epidemic of late-blight.

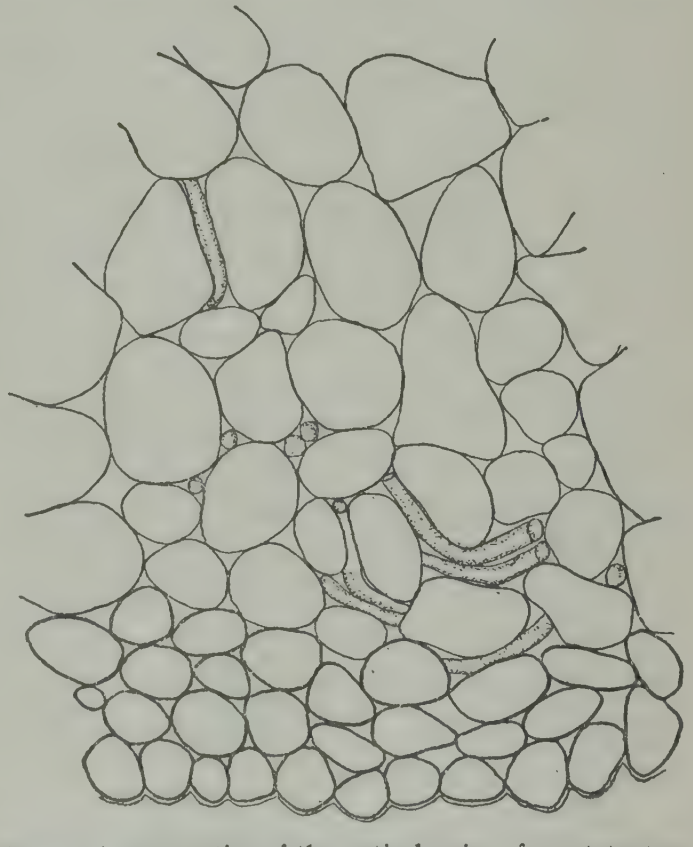

FIG. 3.—A cross section of the cortical region of a potato stem, showing the mycelium of *Phytophthora infestans*. This plant became infected by the mycelium spreading up the stem from the infected parent tuber. This is an early stage of infection, and the tissues of the cortex have not been killed.

The development of late-blight under field conditions was again followed in the fall of 1913 at Houlton, Me. Careful watch was kept on several fields in that vicinity. The first infection by *P. infestans* was found in the field on August 8, following a few days of wet weather. By going through nine different fields six other centers were found. One typical case will serve to illustrate the prevailing conditions at each center. The infected leaves were always the lower ones of the plant. At the center of the infected area the infections were much more numerous than elsewhere, probably about ten times as numerous. These centers of infection varied from 8 to 40 feet in diameter. If the centers had not become too large, a hill could usually be found that was nearly killed and which suggested strongly that it was the point where the primary infection originated. From August 15 to 28 the weather was hot and dry, and during this period the fungus made little headway. On the date last named a rain fell which facilitated the spread of the disease and caused it to become general though not markedly destructive in the

fields not sprayed with Bordeaux mixture; in other words, epidemics were started from the small areas found early in the season. The spread of the disease was wholly comparable to the above-described developments on the small plot at Madison.

Last summer (1914) three similar infection centers were found in fields near Presque Isle, Me. Such a center is shown in Plate VIII, figure 2. The infected area is set off by a white line. The question naturally arises as to how these centers come into existence. Are they due to the planting of infected seed potatoes or to wind-blown spores? It is impossible to answer either of these queries positively, but in the light of evidence now at hand both are probable. There can be no doubt that seed potatoes infected with *P. infestans* are planted by the growers. This has been observed many times, and in one case 46 seed pieces infected with late-blight were taken from a single barrel of seed potatoes which were about to be planted. None of these were badly infected, but such specimens are more certain to produce infected progeny than those badly diseased, as the latter often rot in the ground.

It may well be, therefore, that these infected centers originate from the infected seed, even although the originally infected shoot is not found. This is probably due to its rapid death after the mycelium reaches the surface of the soil. It soon dries up and leaves little evidence of its presence behind. On the other hand, it is easy to understand how these infected centers might be caused by wind-blown conidia, but it is more difficult to explain their origin without making use of the progeny of infected seed tubers. Although it is not definitely shown how these infected centers originate, in the case of the experimental plots it was clear that they came into existence at the same time that the infected shoots developed. It is also known that seed potatoes infected with *P. infestans* are planted.

RELATION OF THE MYCELIUM IN THE SEED TUBER TO THE PROGENY

Logical as it seems that the shoots and plants produced by diseased tubers should become infected in the same way as the young sprouts, such has not been found to be the case by a large majority of the students of this problem. That the mycelium in the diseased tuber may renew infection from one year to another was first supported by experimental evidence in 1861 by De Bary (1). His evidence, however, was not generally accepted, and in 1876 Pringsheim (29) advanced the alternate-host theory. It should be recalled in this connection that De Bary announced the fundamental rediscovery of heteroecism in *Puccinia graminis* in 1865, which probably influenced Pringsheim (29) and many others in accepting the alternate-host theory as a possibility in *Phytophthora infestans*, where oospores were unknown and infected tubers failed to produce infected plants.

Pringsheim's theory, it must be conceded, won some consideration at the hands of practical growers. This is well illustrated in an early paper by Farlow (11) and an article by Jenkins in 1874 (13). The latter discusses 100 reports made by potato growers on the potato fungus. It is very apparent from these articles that clover or straw was thought by many to be an alternate host for *P. infestans*. This theory, as well as others equally fictitious, was not expounded after 1876, when De Bary published his second paper (4) on this subject. At this time he submitted further evidence supporting the perennial-mycelium theory.

De Bary's theory was not confirmed until about 26 years later, when Jensen (14) repeated De Bary's experiments and obtained infected plants which later became centers of secondary infection. He, like De Bary, worked only in the open, where accidental infection by conidia or by oospores is always possible and where such conditions as moisture and temperature are variable factors. In other words, the technique used by Jensen was no more refined than that used by De Bary 26 years earlier; and he, like De Bary (4), was unable sufficiently to define his method so that his results might be duplicated. In view of this fact it is not surprising that Jensen's researches failed to materially strengthen the perennial-mycelium theory.

During the last 25 years repeated efforts have been made by Boehm (6), Smorawski (32), Hecke (12), Clinton (8), Massee (20), Pethybridge (25), and Jones (17) to grow such diseased plants as were described by De Bary and Jensen from infected seed tubers, both under glass and in the open, but little confirmatory evidence has been obtained. This fact, coupled with the very important discovery by Jones (15), Clinton (9), and Pethybridge and Murphy (27) of resting spores borne by the late-blight fungus in pure cultures, has made the perennial-mycelium theory seem even more questionable. This feeling is liberally expressed by Clinton (8).

The fact that so many students have failed to show the relation of infected seed potatoes to epidemics of the disease may well be due to one or all of three factors: (1) Stage of activity of the tuber, (2) temperature, and (3) moisture of the air and soil.

It is well known that the tuber requires a rest period before it will begin to germinate. If an infected tuber is planted in moist, warm soil before this period has elapsed the tuber rots quickly, owing to the activity of *P. infestans* and soft-rot organisms. If, on the other hand, diseased tubers are held in cold, dry storage until late in the winter or early in the spring and then planted, the tuber makes considerable growth before it is overrun by *P. infestans* and soft-rot organisms. In several of our northern potato-growing sections potatoes are stored at temperatures ranging from 0° to 10° C. until only a short time before planting. The fact that *P. infestans* and soft-rot organisms make little or no growth at

this low temperature explains clearly how infected tubers are able to survive the winter season and are in a condition to make rapid growth when placed in the soil. The statements that tubers infected with *P. infestans* very largely rotted in the ground and that a large majority grew and produced normal plants are both very prevalent in the literature, and the author reports similar experiences in his own experiments. These discrepancies, however, may well have been due to the conditions under which the tubers were stored and their state of germination at planting time. Of course, as will be shown later, the influence of moisture and temperature after planting plays an important rôle.

From infected seed tubers growing rapidly the greatest number of infected sprouts and shoots were obtained in a saturated atmosphere at a relatively high temperature (23° to 27° C.). A temperature of 27° seemed even more favorable than 23° C. This is of interest in view of Vöchting's (35) results to the effect that the optimum for the growth of the potato tuber is about 27° and is not out of harmony with the optimum fixed by Jensen (14) for the growth of the mycelium in the potato tuber. How the fungus spreads in the stem and sprout tissues at temperatures between 23° and 27° C. has been described in an earlier part of this paper. The fungus not only traveled up the stem rapidly but also sporulated profusely at such temperatures. In a paper not yet published it is also shown that the growth of liberated zoospores is more rapid at 23° to 24° C. than at lower temperatures. This is true also where the vines have been inoculated with conidia and zoospores. Although no experiments have been made to establish the optimum for the growth of the mycelium in the diseased tuber, the data cited above show that the mycelium is very active at 23° to 27° C. Whatever may be the optimum for the mycelium in the tuber, this point is clear: That temperatures between 23° and 27° C. are more conducive to the growth of the mycelium than lower temperatures, other conditions being favorable.

Although the state of germination of the tuber and the temperature are important, they do not take precedence over moisture. It need hardly be mentioned that *P. infestans*, by virtue of its phylogeny, is a moisture-loving fungus. To the practical grower it is well known also that an epidemic of late-blight need not be feared in a dry season, while in our northern potato sections a wet season is a sure sign of such an epidemic. The mycelium grows very slowly and absolutely refuses to fruit in a dry atmosphere. It has been shown that the spread of the mycelium is materially retarded when tubers infected with *P. infestans* are buried in dry soil. Again, the necessity of moisture is well illustrated in the case of the isolated plantlet referred to. The fungus made little progress in the stem even after reaching the surface of the soil, and it was only by restoring a moist atmosphere that the fungus fruited. It has also been shown that a greater number of the infected

tubers produced young plantlets when they were allowed to sprout in comparatively dry soil.

De Bary (4) describes a case which is interesting in this connection and serves to emphasize the importance of moisture conditions. A potato plant was found which had become infected by *P. infestans* in the parent tuber. Portions of the stem just above the surface of the soil were infected and discolored, but dry weather prevented the fungus from progressing farther in the tissues or sporulating. This was surely a case where moisture checked the fructification of the fungus. Two similar cases, which are even more striking as showing the close relation of moisture and development of the fungus, are described in this paper. In these the fungus grew up the stem to the surface of the soil and infected the foliage, but the hot, dry weather checked its further spread.

It is not necessary that the optimum conditions for the growth of the fungus should prevail continuously. This is clear from the author's experiments where the tubers were started in dry soil and later transferred to wet soil and the fungus grew up the stem. Too much emphasis can not be placed upon the importance of environmental factors and the state of germination of the tuber in the production of diseased plants from seed infected with *P. infestans*. A combination of these three conditions is not always prevalent in the open nor in the ordinary greenhouse, which may well account for the accumulation of negative data. In this connection may be cited one of several experiments where over 300 tubers were planted in a greenhouse, where the moisture and temperature could not be readily controlled, and not a single infected plant was obtained. Clinton (8), Pethybridge (24, 25), and many others have reported similar results from extensive field trials.

In closing this portion of the discussion it should be pointed out that not all infected tubers give rise to infected shoots and become centers of foliage infection. In fact, only a small proportion function in this way, according to the studies of the author; nor has any method been worked out whereby an infected tuber can be made to give rise to infected plants such as are shown in Plates VI and VII. Whether the progeny of a diseased tuber will or will not become infected is determined by the response of the fungus and host, coupled with environmental conditions. It is known beyond all possibility of doubt, however, that a certain proportion of the diseased tubers planted under field conditions may produce progeny which becomes infected by the mycelium growing up the stem. Once above the surface of the soil, the fungus may sporulate and cause foliage infection on the initial and adjoining hills. Infection spreads rapidly from such an infection center and is the forerunner of an epidemic. Hecke (12) has also noted this early stage in the development of an epidemic. It seems logical to assume that these infection centers start from planted infected seed potatoes.

This method of perpetuation readily explains how *P. infestans* has spread from its native home in South America to every corner of the globe. As pointed out by Jensen (14), it was probably brought to Europe in the mycelial stage in seed potatoes. Likewise, it may well have gone to Australia, New Zealand, North America, and other parts of the world.

MYCELIUM OF PHYTOPHTHORA INFESTANS IN THE SOIL

That the mycelium might live over winter in the soil was possibly first suggested by Kühn (18), who arrived at this assumption because he was unable to grow infected plants from diseased tubers, combined with the fact that the potato fungus occurred year after year. This theory received support later at the hands of Brefeld (7) in connection with his excellent cultural studies of the smuts. He devoted some attention to *P. infestans* also and was probably the first to grow this fungus saprophytically in semipure cultures. It was this significant achievement that led him to support Kühn's theory.

Darnell-Smith (10) has studied the possibility of *P. infestans* living over in the soil. A large number of experiments were made by mincing infected tubers in the soil and planting it to potatoes. He also smeared spores on the tubers when planted, but in no case did he get any infection of *P. infestans*. Some recent experiments by Stewart (33) also bear directly on Brefeld's theory (7, p. 26). He planted healthy tubers in soil mixed with blighted vines and tubers and made conditions highly favorable for the infection of the growing potato plants. No infection of *P. infestans* was obtained.

According to the writer's studies, under certain conditions of moisture and temperature the fungus may grow and sporulate on the surface of the soil to a very limited extent, as described in an earlier part of this paper, but no evidence was obtained showing that it remains alive in the soil for extended periods of time. Jones, Giddings, and Lutman (17) have also recorded the fact that the fungus may spread from infected tissue out over the surface of the soil to a limited extent. Our increased knowledge of culturing parasitic fungi on artificial media, and especially of *P. infestans*, does not permit such deductions at the present time as were made earlier by Brefeld (7).

MASSEE'S LATENT-MYCELIUM THEORY

The early literature on *P. infestans*, then known as the "potato murrain," is full of interesting theories as to its origin. The literature is in every case naturally tinted with spontaneous generation and lack of information as to the life history of the fungus. Fully as interesting is a theory more recently advanced by Massee (20). He maintains that the usual explanation for the sudden appearance of *P. infestans* over wide areas by the dissemination of conidia is inadequate and that the fungus is

latent in apparently healthy potato plants. It is, of course, obvious that Massee makes two radical departures from well-established principles: First, that the rapid dissemination of spores is not sufficient to cause an epidemic; and, second, that mycelium remains latent in the potato tissues.

The development of an epidemic by means of conidia under field conditions has been carefully followed and described in an earlier part of this paper, and the results fully confirm Ward (36) and others. That conidia or asexual spores are able to cause epidemics in the case of a great number of parasitic fungi is well known and needs no further argument. Had Massee demonstrated histologically the presence of latent mycelium in the apparently healthy potato plant as a whole, the latent-mycelium theory would have been worthy of more careful consideration.

WILSON'S SCLEROTIA-LIKE BODIES OF THE POTATO FUNGUS

Another singular theory to account for the perpetuation of *P. infestans* is that proposed by Wilson (37). He believed he had found sclerotia-like bodies on the potato tuber and plant as a whole which were the resting organs of the potato fungus. This theory was later indorsed, strangely enough, by Plowright (28) and W. G. Smith (31). The latter stated that it was his conviction that the bodies Wilson found were of fungous origin, and possibly those figured by Martius (19). These sclerotial bodies were later proved by Murray and Flight (22) to be calcium-oxalate crystals.

Later Wilson (38) reported a more fictitious discovery, that of a mucoplasm existing in the potato plant, which was able to give origin to late-blight.

CONIDIA BORNE IN THE SOIL RENEWING INFECTION

De Bary early suggested that the fungus might perpetuate itself by means of the conidia, although he considered it very improbable that primary infection often, if ever, takes place in this way. Jensen (14) claims to have found a case where the shoots were killed before they reached the surface of the soil, and the spores on these shoots infected the stem of a healthy plant growing in close proximity. Clinton (8) also cites a case where conidia borne under wet cotton possibly functioned in causing infection in one of his pot cultures. In this paper are recorded further experiments showing that the fungus fruits with great ease on the cut surfaces of the seed tuber and on infected sprouts in the soil, although so far no case has been found where such spores functioned in producing infection above the surface of the soil. It is not impossible, however, that it might happen, and Hecke (12) records such a case.

As stated above, it is not improbable that spores produced on the cut surface of diseased tubers or sprouts may cause infection in some cases, yet the author can not hold with Hecke (12) and Clinton (8) that primary infection due to conidia occurs uniformly throughout a field. In an

earlier part of this paper it is shown how an epidemic developed by artificially inoculating two plants in a plot of potatoes in a section of the country where *P. infestans* did not develop that year and how plants immediately surrounding the two initially infected ones succumbed before any of the others at a greater distance, thereby giving rise to infection centers in the plot in which the vines were killed long before the rest and which increased until it included the whole plot.

Other cases are cited where similar centers known to have originated from the spread of the mycelium up the stem were found and carefully watched under field conditions during the growing seasons of 1913 and 1914. Furthermore, the development of *P. infestans* has been followed for the last three seasons, but no evidence has been obtained to show that it originates uniformly on the lower leaves throughout a whole field. In many cases, when observations are made early enough, the disease is found to originate at some one point and spread outward and radially.

RESTING SPORES OF PHYTOPHTHORA INFESTANS

Resting spores, or oospores, are produced by most of the species of Peronosporaceae. Their function, as is well known, is to bridge the fungus over periods unfavorable for its growth and development. Whether *P. infestans* has oospores has been a bone of contention for the last 60 years. Until recently, however, the prevailing opinion has been that oospores were not produced by this fungus.

During the last decade bodies resembling oospores have been found in pure cultures by Jones (15), Clinton (9), and Pethybridge (26). This discovery has doubtless influenced Pethybridge (25, p. 343) in making the following statement:

> It appears to be practically certain that the primary attack of blight each season is due to *spores*, but where these spores come from is not known with certainty, and whether they are similar to those produced on the potato foliage in warm, moist weather in the summer after the primary infection of the crop has taken place, or are of the nature of the thick-walled resting spores produced by species of Phytophthora allied to *Phytophthora infestans*, can not definitely be stated at present.

This statement plainly discredits the perennial-mycelium theory and suggests that spores, either conidia or oospores, function in renewing infection. That the mycelium in diseased seed tubers may renew an epidemic of late-blight has been clearly shown in an earlier part of this paper and needs no further argument.

Pethybridge (25) unfortunately does not define the spore that serves to perpetuate *P. infestans*. If he means conidia, there is little evidence to support his contention, as has already been pointed out. On the other hand, it must be conceded that the discovery of bodies resembling oospores in pure cultures of *P. infestans* must be seriously considered when discussing the overwintering of the fungus. At present, unfortunately, there is little positive evidence to support the oospore theory.

It is to be hoped that the recent researches on this problem will afford an angle of approach that will yield positive evidence in the near future.

In closing it should be pointed out that, although *P. infestans* rarely produces oospores in the potato plant, this should not be looked upon as abnormal. As shown in this paper, the production of resting organs is not necessary for the hibernation of the fungus. The mycelium is quite sufficient. There are many species closely related to *P. infestans* that produce few resting spores on certain of their hosts. These may perpetuate themselves from one season to another by means of the living mycelium in the perennial parts of the host plant in much the same way as already described for *P. infestans*. The sparing production of oospores and the hibernation of the mycelium are therefore not uncommon in several species of this family.

SUMMARY

It is clear from the author's experiments that the mycelium of *Phytophthora infestans* spreads in the tissues of the potato tuber and finally reaches the sprouts. The growth of the fungus is retarded when diseased tubers are held in dry soil or at temperatures below 5° C. Infected tubers rot rapidly when placed in warm wet soil. This explains the wide variation in stand obtained by earlier writers. A temperature of 23° to 27° C. and a well-watered soil were found to be the most favorable for the mycelium to spread in the tuber and grow out into the sprouts, both when partially and when wholly covered with soil. Under these conditions the sprouts may become infected from 4 to 20 days after planting, regardless of their size and age. The time required is doubtless influenced by the proximity of the mycelium to the buds and the external conditions.

The mycelium of *P. infestans* may remain alive in seed tubers planted in the soil for at least 45 days, and it is very possible that under conditions less favorable for the soft rots which follow *P. infestans* in the tuber the fungus may live much longer. None of the author's results or observations tend to show that the potato fungus is *latent* in the stems and leaves of plants growing from diseased tubers, as stated by Massee (20).

Laboratory tests showed that the fungus infects not only the sprouts but also the shoots that break through the soil. The mycelium grows from the tuber into the stem, where it travels up to the surface of the soil and sporulates, as held by De Bary (4) and Jensen (14). This usually takes place in the small dwarfed shoots in a hill.

Potato tubers infected with *P. infestans* used for seed purposes and planted under field conditions may cause the development of an epidemic. The mycelium grows from the parent tuber up into the stem exactly as shown in the laboratory experiments. It later sporulates and foliage infection results. Ten such cases were found and followed

in northern Maine during the growing seasons of 1913 and 1914. All except two of these became centers for foliage infection, and severe epidemics of *P. infestans* followed.

Conidia of *P. infestans* may be borne on the cut surfaces and sprouts of tubers when planted under field conditions. As the cut surface corks over or the tuber decays, the fructification of the fungus decreases. Spores taken from tubers two to three weeks after they were first planted showed only limited germinating capacity. No evidence was obtained tending to show that the conidia borne in the soil are instrumental in starting foliage infection.

The mycelium of *P. infestans* spreads most rapidly in the cortical tissues of the stem, where it travels up more rapidly than down.

Epidemics of late-blight may start from a single shoot or hill naturally or artificially infected with *P. infestans*. The infection spreads radially from the initial point of infection during the early stages of the development of an epidemic. These spots of infection in the fields probably come into existence through the planting of seed potatoes infected with *P. infestans*.

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PLATE IV

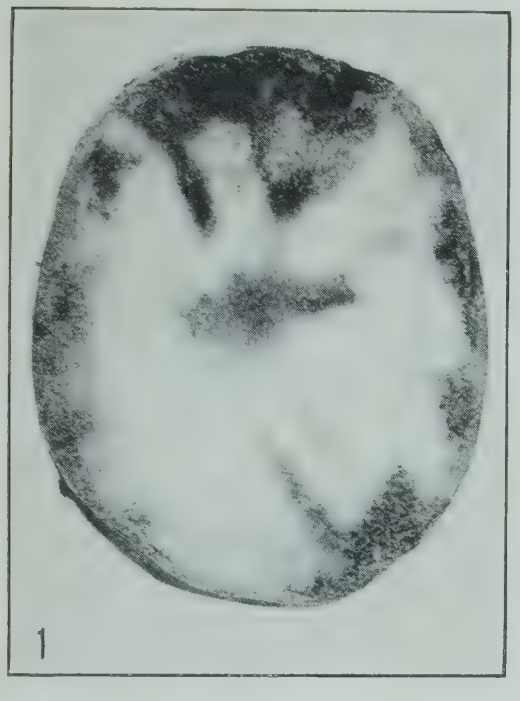
Phytophthora infestans: Infection of potato tubers

Fig. 1.—Cross section of a tuber which was infected with *P. infestans* and was planted in the greenhouse in rather dry soil. After two months it was dug up and found to be firm and containing living mycelium of the fungus.

Fig. 2.—This tuber was inoculated at the eye surrounded by the paraffin ring. The mycelium ran through the tissues and grew out into four of the sprouts at the bud end of the tuber.

Fig. 3.—Cross section of an infected tuber planted in sterilized soil in the greenhouse which developed a shoot that became infected through the parent tuber.

Fig. 4.—The small stunted shoot which grew from this infected tuber shows the progressive discoloration caused by *P. infestans* growing up the stem.



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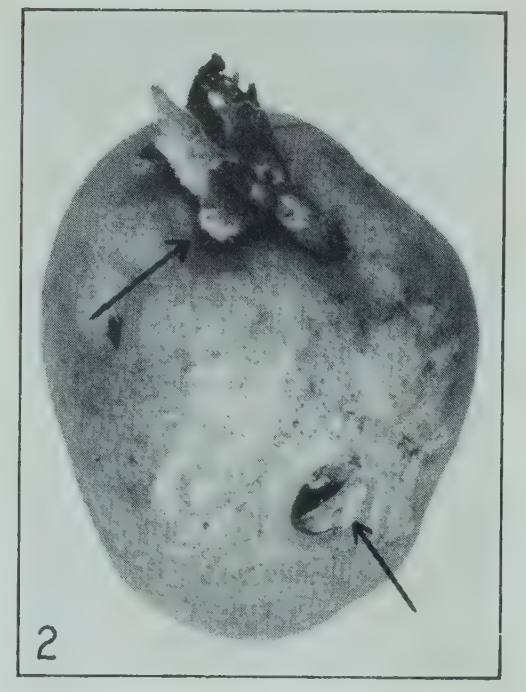

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JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V. WASHINGTON, D. C., OCTOBER 18, 1915 No. 3

ENZYMS OF APPLES AND THEIR RELATION TO THE RIPENING PROCESS

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INTRODUCTION

Several years ago the writer, at that time connected with the Washington State Agricultural Experiment Station, in cooperation with Mr. N. O. Booth, the horticulturist of that Station, undertook an investigation of the possibilities of slowing up the ripening of fruits by means other than cold storage. While these investigations were in progress, Mr. Booth severed his connection with the Station, but it was understood that he would continue the studies in his new location. For that reason no report of our observations at that time has ever been published; but, since no publication of the results of further work along this line has appeared, the writer feels at liberty to assume that the investigation has been discontinued and to discuss briefly the observations which were made, since they form the starting point for the studies to be reported in this article.

Since the term "ripening" is used to designate various different stages in the development of fruit, it is first necessary to define it as it will be used in this article. Seeds upon ripening usually lose water and go into a resting stage from which germination may take place. But the flesh of an apple (*Malus* spp.) or similar fruit has no definite connection with the life history of the embryo of the seed; hence, its "ripeness" can not be measured in terms of the germination ability of the seed. The fruit itself goes through the following stages of development. There is first a period during which the fruit is growing—i. e., increasing its weight of dry matter. At the end of this period, no matter whether the fruit remains on the tree or is picked off, growth ceases and chemical changes set in which result in the development of the characteristic odor and flavor and later in the disintegration of the flesh of the fruit. When this disintegration proceeds far enough, the fruit becomes soft, mushy, or overripe, and usually at either this or some preceding stage organisms of decay gain entrance to the tissues, and the fruit rots. In the absence

of infection with any germs of disease or decay, the fruit loses water and shrivels up to a withered mass. The group of changes that take place during the second of these stages—i. e., the period between the cessation of growth and the disintegration of the tissue until it becomes soft or mushy—will be termed the "ripening process."

The object of all storage or preservation of fresh fruit is to slow up the ripening process and so to prolong this period as much as possible. It is a well-known fact that temperature has an important effect upon the rapidity with which these changes take place. It was the object of the studies referred to above to determine whether other factors also influence the rate of these changes and whether they are due in part to infection with disease germs or are wholly enzymic in character.

Two general methods of study were attempted. First, an attempt was made to surround individual apples with a film or coating which would prevent gaseous exchanges and bacterial infection. Repeated efforts to secure a perfect film of this sort with a variety of different materials proved failures; so this method was abandoned. The second method involved the sealing up of the apples in atmospheres of different pure gases under as nearly sterile conditions as possible in order to prevent both disease infection and the ordinary gaseous exchanges. Several large glass bottles, each capable of holding about a peck of apples, were fitted with tight stoppers provided with a glass inlet tube reaching to the bottom of the bottle and an exit tube extending just through the cork. Carefully washed apples were rinsed in a dilute solution of formaldehyde, followed by distilled water, and immediately introduced into the jars and the stoppers sealed in. The apples were of the Alexander variety and were almost ripe—i. e., they would only keep a few days longer without becoming soft. After sealing in the stopper the inlet tube was connected to a supply of pure gas and the latter passed through until no air could be detected in the gas issuing from the exit tube, when the glass tubes were melted off, thus effectively sealing the jars. This method did not, of course, remove the air contained in the tissues of the apples themselves, but this was relatively small in amount.

Each of five jars was filled with one of the following gases: Hydrogen, nitrogen, oxygen, carbon dioxid, and sulphur dioxid; a sixth was sealed with its ordinary air content. No moisture-absorbing material was placed in the jars, as it was thought that this would produce abnormally rapid losses by evaporation from the tissues of the fruit. Further, the recognized chemical changes in the fruit during the ripening process are probably not influenced by the moisture content of the surrounding air, so that the saturation of the air in the jars with water vapor evaporated from the fruit would not be likely to influence the nature of these changes, while constant absorption of this vapor would mean rapid shriveling of the fruit.

The jars were left in a warm, light laboratory and were examined from time to time. The apples in air continued to ripen normally and

in about four weeks were visibly overripe, the lower ones beginning to collapse under the pressure of the weight of the upper layers. Those in oxygen seemed to ripen a little more rapidly, but the difference was not nearly so great as had been expected and was hardly enough to warrant any conclusion that pure oxygen hastened the ripening process. Those which were surrounded by nitrogen and hydrogen did not soften so noticeably, but became discolored and unhealthy in appearance, a phenomenon later observed and reported by Hill (8).[1] After some 8 or 10 weeks, however, these apples also softened into a mushy mass. The apples in carbon dioxid and in sulphur dioxid remained apparently firm and unchanged for a long time, except that the latter gas completely bleached the skins of the apples in its jar, leaving them a uniform creamy white in color. After nearly six months had elapsed, these jars were opened and the fruit examined. That which had been in an atmosphere of sulphur dioxid was firm and solid, but was, of course, so thoroughly impregnated with the disagreeable gas that its quality could not be judged. The apples which had been in carbon dioxid were firm in flesh, possessed the characteristic apple odor, although the gas in the jar had a slight odor of fermented apple juice, and were not noticeably injured in flavor.

It appeared, therefore, that the phenomena ordinarily associated with ripening were greatly inhibited by an atmosphere of carbon dioxid, but that the cause of this inhibition was not wholly a lack of oxygen. It seemed that the changes taking place in the apple were not simple respiratory changes, but probably in large part were internal enzymic activities.

The experiment was repeated the following summer, using raspberries, blackberries, and loganberries instead of apples. It was found that berries which softened in 3 days in air would remain firm for from 7 to 10 days in an atmosphere of carbon dioxid. At this point the studies were interrupted by a change in professional engagements and have not been resumed.

Recently, Hill (8) reported a series of observations so similar in character that interest in the matter was revived; and opportunity being presented for a systematic study of the enzyms of apples by a graduate student[2] working under the writer's direction, such a study was undertaken, with the results reported below.

CHANGES IN CHEMICAL COMPOSITION OF APPLES DURING RIPENING

The changes in the chemical composition of apples during ripening have been very thoroughly studied by Bigelow, Gore, and Howard (2). The report of their investigations contains a careful review of the literature on the subject, together with significant contributions from the

[1] Reference is made by number to "Literature cited," p. 116.

[2] The writer's thanks are due to Miss Inez Everett, the graduate student who assisted in the preparation of the material for examination and the carrying out of the several tests.

work of the authors themselves. Briefly summarized, the results of these investigations show that the principal changes which take place in the apple during ripening are as follows:

(1) A slight but continuous decrease in total acidity calculated as malic acid.

(2) A gradual decrease in sucrose.

(3) A gradual increase at first, followed by a later slight decrease, in invert sugar and total carbohydrates calculated as invert sugar.

(4) The disappearance of starch early in the ripening process.

ENZYMS IN APPLES

The literature which is available to the writer contains very few references to any investigations of the enzyms that are present in apples.

Lindet (9) found in the juice of apples a soluble ferment which causes coloration of the tissues by the absorption of oxygen and the giving off of carbon dioxid, which is inoperative when the juice has been boiled, which may be precipitated from the juice by alcohol, and which oxidizes pyrogallol to purpurogallin. He concluded that the coloration is due to oxidation of tannin by a soluble ferment of the kind designated by Bertrand as laccase (now called "oxidase").

Warcollier (12) is the only other author who reports work on enzyms in apples. Although he was unable to find invertase in apple juice, he believes that it must be present in order to account for the apparent inversion of sucrose during the ripening process. He suggests that the enzym may have been retained by the apple marc and consequently may have escaped his observation.

The meagerness of the work which has been done along this line is probably due to the fact that the flesh of the apple is not an important element in the physiology of the plant's growth and has little scientific interest to students of plant physiology or biochemistry. But its economic importance and the desirability of knowledge concerning the ripening process as a factor in the storage of perishable fruit products are apparent and, in the writer's opinion, fully justify a thorough study of the subject. The present paper does not constitute an exhaustive report. It does not include, for example, a comparison of enzymic activity of rapidly maturing varieties of apples as contrasted with those which ripen more slowly and, hence, are better keepers. It is believed, however, that the facts here presented will serve as a foundation for such further work as may be found desirable.

EXPERIMENTAL WORK

The apples used in these investigations were secured from an orchardist in the State of Washington and were of varieties known to be good keepers—i. e., slow in ripening in storage.

PREPARATION OF MATERIAL FOR EXAMINATION

The first problem was naturally that of securing an extract of the cell contents of the apple pulp which would contain the enzyms in active form. Since it was not known whether any or all of these enzyms would be diffusible through the cell walls (extracellular), a preliminary mechanical rupturing of the cells or rendering of them permeable by drying, according to well-known methods of technique in enzym study, was necessary. Several methods were tried, as follows:

(1) Whole apples were run through a horse-radish grater and the resulting pulp pressed in an ordinary laboratory hand press. The resulting juice was thick, with small particles of pulp, and attempts were made to clarify it by filtration. These were unsuccessful because of the clogging of the filter by the pectin bodies of the juice.

(2) Apples were rasped and pressed as before and the juice allowed to stand for some time, during which the suspended solids settled fairly well, and the supernatant clear juice was decanted. Precautions against enzymic activity during the settling were taken by keeping the settling jars in an ice box.

(3) An attempt was made to secure a dry powder of the apple pulp by drying thin slices in a vacuum desiccator over sulphuric acid; but the large proportion of sugars and pectin bodies in the tissue made this impossible, the slices being gummy and impossible to grind into a powder even after six weeks' exposure in the desiccator.

(4) Thin slices of apple pulp were treated by the acetone-ether method first used by Buchner, Albert, and Rapp (1) in the preparation of *Dauerhefe*, or active dry yeast powder. This process was very satisfactory, the apple slices, after the treatment and exposure to the air overnight, becoming so dry and brittle that they could easily be powdered between the fingers and very easily reduced to a fine powder in a mortar. Several investigators have reported that the enzymic activity of the dry powder so prepared is not less than that of the original tissue, and the writer's observations confirm this. This appears to afford an excellent means of preparation of sugary or gummy materials of this kind for enzym extractions.

(5) Apples were peeled and cored, and the flesh cut into small blocks. These were then mixed with an equal weight of sharp quartz sand and the mixture rubbed gently in a mortar until uniformly disintegrated. The mixture was then transferred to a fine silk cloth and pressed gently. By this means a limpid juice could be obtained which was nearly free from pectin materials, although slightly cloudy with suspended particles of pulp. Experience has shown that harsh grinding and severe pressure result in diminished activity of the juice, particularly in its oxidase activity, but with gentle manipulation, as above, very active juice can be obtained.

(6) A quantity of concentrated apple juice prepared by Gore (6) by his freezing method was secured and used in some of the tests, since it was thought that this process would be likely to leave the enzyms uninjured in the juice.

EXAMINATION OF DIFFERENT PREPARATIONS FOR ENZYMS

In the earlier examinations reported below, several different preparations were examined simultaneously for the particular type of enzym which was being sought, in order to avoid any wrong conclusion from improperly prepared material. Experience soon showed, however, that either the acetone-dried powder or the pulp ground with quartz sand would yield active extracts in every case where activity could be found in material prepared by any of the above methods, and one or the other of these two preparations was used in all the later tests. The acetone-dried powder has the advantage that a considerable quantity of material can be prepared at one time for subsequent examination.

DIASTASES

Diastases have been shown by Thatcher and Koch (11) to be readily diffusible into water surrounding cell tissues. It seemed probable, therefore, that if enzyms of this type were present in apple flesh they would appear in juice expressed from pulp after thorough rasping. Samples of clear juice by decantation were secured from three different varieties of apples and tested for diastatic activity. Four separate mixtures were prepared for each variety of juice. The first contained 10 c. c. of a 10 per cent solution of soluble starch prepared by the Lintner method (5), 10 c. c. of the juice in question, and 10 c. c. of distilled water. The second contained 10 c. c. of soluble starch, 10 c. c. of the juice which had been boiled for 10 minutes and made to its original volume with water, and 10 c. c. of distilled water. The third contained 10 c. c. of soluble starch, 10 c. c. of the unboiled juice, sufficient *N/10* sodium hydroxid (NaOH) to exactly neutralize the juice used (determined by a preliminary titration, using phenolphthalein as indicator), and enough distilled water to make the total volume 30 c. c. The fourth, or control, contained 10 c. c. of soluble starch and 20 c. c. of distilled water. The contents of each flask were thoroughly mixed and an aliquot drawn off for the determination of reducing sugars present in the solution. The flasks containing the remainder of the solution were then placed in an incubator for 30 minutes at 40° C., these being the conditions recommended by Sherman, Kendall, and Clarke (10) for all determinations of diastatic activity. At the expiration of this period action was stopped by adding sufficient *N/10* sulphuric acid to make the total volume a *N/200* solution, and an aliquot equal to that taken before the digestion was drawn off for the determination of its reducing sugar content. The soluble proteins were precipitated and the reducing sugars determined by the method out-

lined in the article by Thatcher and Koch (11). The results obtained are given in Table I.

TABLE I.—*Results of tests for diastase in the flesh of apples*

Variety and material.	Reducing sugars.	
	Before action.	After action.
	Gm.	*Gm.*
Jonathan:		
Decanted juice	0.0192	0.0183
Decanted juice (boiled)	.0207	.0212
Decanted juice (neutralized)	.0197	.0192
Control (water only)	None.	None.
Yellow Newtown Pippin:		
Decanted juice	.0113	.0113
Decanted juice (boiled)	.0217	.0212
Decanted juice (neutralized)	.0103	.0113
Control	None.	None.
Rome Beauty:		
Decanted juice	.0103	.0098
Decanted juice (boiled)	.0207	.0202
Decanted juice (neutralized)	.0113	.0103
Control	None.	None.

At a later date, when other preparations of apple material were available, tests were made of the reducing sugars present in equal aliquots of soluble-starch solution which had been digested for 30 minutes at 40° C., with both boiled and unboiled extracts of these materials, with the results given in Table II.

TABLE II.—*Results of tests for diastases in various preparations made from the flesh of apples*

Material.	Reducing sugars found after action.	
	Active extract.	Boiled extract.
	Gm.	*Gm.*
Water extract of acetone-dried pulp	0.0202	0.0207
Juice concentrated by Gore's process	.0356	.0351
Juice from pulp ground with quartz sand	.0316	.0316

From these results it is evident that the juice contained no diastases. It appears, therefore, that after the starch disappears from the apples the diastases also disappear. None of the apples which were available for these investigations contained any starch.

INVERTASE

Invertase was tested for in two samples by a method precisely like that used for diastases except that 10 c. c. of a 10 per cent solution of sucrose were used in place of the soluble starch. The results obtained are given in Table III.

TABLE III.—*Results of tests for invertase in the flesh of apples*

Variety and material.	Reducing sugars.	
	Before action.	After action.
Yellow Newtown Pippin:	*Gm.*	*Gm.*
Decanted juice	0.0113	0.0113
Decanted juice (boiled)	.0212	.0217
Decanted juice (neutralized)	.0113	.0103
Control (water only)	None.	None.
Rome Beauty:		
Decanted juice	.0098	.0103
Decanted juice (boiled)	.0202	.0207
Decanted juice (neutralized)	.0103	.0113
Control	None.	None.

These results being so contrary to what was expected, it was thought best to use material prepared for examination in several other ways in testing for invertase. Accordingly, a water extract was made of some acetone-dried powder from Rome Beauty apples, another sample of the same apples was ground with quartz sand and its juice expressed, and finally a sample of the Gore's concentrated apple juice was diluted to about the same concentration as normal apple juice. Each of these materials was then incubated with sugar solution in the usual way, using unboiled and boiled samples of both the acid and neutralized juice in each extract. The reducing sugars found in the digested mixture from the unboiled or "active" extract and from an equal aliquot of boiled extract are given in Table IV.

TABLE IV.—*Tests for invertase in various preparations from the flesh of apples*

Material.	Reducing sugars found after action.	
	Active extract.	Boiled extract.
	Gm.	*Gm.*
Water extract of acetone-dried pulp	0.0207	0.0207
Juice concentrated by Gore's process	.0396	.0396
Juice concentrated by Gore's process (neutralized)	.0376	Lost.
Juice from pulp ground with quartz sand	.0192	.0187
Juice from pulp ground with quartz sand (neutralized)	.0192	.0192

The results shown in Tables III and IV indicate the absence of any invertase in apple flesh and confirm the observations of Warcollier (12), referred to above. It appears, therefore, that changes during ripening which result in the inversion of sucrose, if they actually occur, must be due to some other cause than the presence of invertase in the apple tissues. The fact that some investigators have not been able to find evidence of this inversion of sucrose during ripening casts some doubt upon its actual

occurrence, there being always the possibility that observed changes in the nature of the sugars present in successive samples may be due to the action of organic acids during the preparation of the samples for analysis.

TANNASE

Determinations of the tannin content of each of the four varieties of apples which were being used by Proctor's modification of Löwenthal's method[1] showed that the flesh of the apples contained the following percentages of tannin: Rome Beauty, 0.208; Arkansas Black, 0.192; Yellow Newtown Pippin, 0.208; King David, 0.132.

It seemed advisable to ascertain, therefore, whether any tannin-hydrolyzing enzym was present in these tissues. Accordingly, a quantity of pulp from each variety was ground with quartz sand and the juice expressed. One portion of the juice from each variety was boiled and another left unboiled. Aliquots of the boiled and unboiled juice were placed in each of two test tubes, to one of which 2 c. c. of a 10 per cent solution of Merck's tannic acid was added, in order to insure sufficient excess of substrate material. The four sets of four tubes each were placed in an incubator at 40° C. for 24 hours. At the end of this time a few drops of a 10 per cent solution of ferric chlorid were added to each test tube and the intensity of color developed in the tubes containing check boiled and unboiled juices was compared. In no case could the slightest difference in intensity of color be observed, from which it was concluded that the juices contained no tannase.

EMULSIN

Glucoside-splitting enzyms were tested for in boiled and unboiled juices prepared from each of the four varieties of apples by digesting aliquots of these juices with 2 c. c. of a 1 per cent solution of amygdalin for 24 hours at 40° C. In no case was any odor of benzaldehyde perceptible at the end of this time, while check tubes to which emulsin was added gave a pronounced odor after only 10 minutes' contact with the amygdalin used. Hence, it was concluded that the apple flesh contains no enzym of the emulsin type.

ESTERASES

One of the noticeable changes in an apple during the ripening period is the development of its characteristic odor and flavor, due chiefly to the ester ethyl malonate. Such esters are usually accompanied in nature by a corresponding esterase; hence, it seemed advisable to test the flesh of the apples for an esterase which would hydrolyze ethyl malonate.

Accordingly, apple juice was obtained by the quartz-sand method and a series of test tubes prepared with the following contents: (1) 5 c.c. of apple juice, 5 c. c. of ethyl malonate, and 10 c. c. of distilled water; (2) 5 c. c. of apple juice which had been previously boiled for 10 minutes,

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p. 1908. See p. 150.

cooled, and made up to its original volume, 5 c. c. of ethyl malonate and 10 c. c. of distilled water; (3) 5 c. c. of apple juice, 5 c. c. of ethyl malonate, sufficient *N/10* sodium hydroxid to render the mixture alkaline in reaction, and enough distilled water to make the total volume the same as in the other tubes; (4), (5), and (6) the same as (1), (2), and (3), respectively, except that a 0.1 per cent solution of steapsin was used in place of the apple juice, as a check upon the reaction conditions. These mixtures were kept in an incubator at 40° C. for 20 hours, after which an aliquot of the mixture was drawn off and titrated with *N/100* sodium hydroxid, using phenolphthalein as indicator, with the results given in Table V.

TABLE V.—*Test for esterases in the flesh of apples*

[Ethyl malonate used as substrate]

Material.	N/100 alkali required.
	c. c.
(1) Apple juice	9.2
(2) Apple juice (boiled)	7.5
(3) Apple juice (with excess of *N/10* alkali)	[a]39.8
(4) Steapsin solution	7.3
(5) Steapsin solution (boiled)	None.
(6) Steapsin solution (with excess of *N/10* alkali)	40.9

[a] In addition to *N/10* sodium hydroxid used to make reaction alkaline.

The data presented in this table clearly indicate the presence in the juice of an esterase capable of hydrolyzing ethyl malonate and similar in its action to steapsin. A slight increase of acidity in test tube (1) over that in (2) indicates a slight hydrolytic action even in the acid medium of the unneutralized juice; while in alkaline medium the activity was almost identical with that of the 0.1 per cent steapsin acting in a similar medium.

OXIDASES

Owing to the fact that Lindet's observations (9) mentioned above, the well-known phenomenon of the coloring of apple tissues when exposed to the air, and the qualitative guaiac reaction for oxidases all point to the presence of active oxidases in apples, a quantitative determination of their presence in the different samples available for this investigation was determined upon. Bunzel (3) has shown the objections to the various methods which have been proposed for the quantitative measurement of oxidase activity by various colorimetric determinations and has perfected a manometric method for the purpose. Correspondence with Dr. Bunzel resulted in his kind permission to make use of his apparatus for the investigation of the materials used in this study. Several samples were accordingly taken to his laboratory and their action toward various oxidizable materials determined according to his method. In carrying out the operation, 0.1 gm. of the acetone-dried powder or 2 c. c. of the apple juice obtained by the quartz-sand method were intro-

duced into one arm of the apparatus, 0.01 gm. of the material to be oxidized placed in the other arm, the proper amount of distilled water added in each arm, and the apparatus placed in the constant-temperature box and allowed to stand for 30 minutes to come to a uniform temperature. The apparatus was then closed, the shaking started, and the manometer readings taken at 15-minute intervals. The final readings, with the kind of material and nature of oxidizable reagent used in each case are given in Table VI.

TABLE VI.—*Oxidase activity of various apple preparations toward different oxidizable reagents*

Variety and material.	Oxidizable reagent.	Time of maximum action.	Diminished pressure.
		Min.	*Cm.*
Rome Beauty:			
Acetone-dried powder	Pyrogallol	45	0.10
Do	Pyrocatechol	60	.60
Do	Guaiacol		0
Do	Tyrosin		0
Yellow Newtown Pippin:			
Acetone-dried powder	Pyrogallol	45	.35
Do	Pyrocatechol	60	1.75
Do	Guaiacol	60	.15
Do	Tyrosin		0
King David:			
Acetone-dried powder	Pyrogallol	60	.20
Do	Pyrocatechol	60	1.45
Do	Guaiacol	60	.15
Do	Tyrosin		0
Arkansas Black:			
Acetone-dried powder	Pyrogallol		0
Do	Pyrocatechol	45	.55
Do	Guaiacol		0
Do	Tyrosin		0
Juice from pulp with quartz sand	Pyrogallol	30	1.45
Do	Pyrocatechol	30	3.50
Juice from pulp with quartz sand(boiled)	Pyrogallol		0
Do	Pyrocatechol		0

These results clearly show that apple pulp and apple juice contain an active oxidase, or oxidases, which accelerate the absorption of atmospheric oxygen by pyrocatechol and pyrogallol, and to a slight extent by guaiacol. The activity toward pyrocatechol is much greater than toward the other reagents, indicating the probability that the tannin of apples, which is so readily oxidized on exposure to air under the influence of the oxidases present, is of the pyrocatechol type.

PROTEASES

Protein-splitting enzyms in the flesh of the apple were tested for as follows: A saturated solution of egg albumin was prepared and 5 c. c. of it were placed in each of three test tubes. In one of these, 5 c. c. of apple juice, prepared by grinding the pulp with quartz sand, were added; to the second, 5 c. c. of the same juice, which had been boiled for 10 minutes,

cooled, and made to its original volume; and to the third, 5 c. c. of distilled water. Another set of three test tubes was prepared with the same proportions of materials, but using a 1 per cent solution of Witte's peptone in place of the albumin solution. The tubes so prepared were kept in an incubator at 40° C. for 24 hours. At the end of this time an aliquot of each mixture was drawn off and the quantity of amino acids present in it determined by the ninhydrin method recently proposed by Harding and MacLean (7), using a solution of glutamic acid containing the equivalent of 0.1 mgm. of nitrogen in the amino-acid form per cubic centimeter for the production of the standard color.

The characteristic color due to amino acids appeared in all the tests except the one in which only water and albumin were used. The amino-acid equivalent in each case, as determined by comparison with the standard color, is given in Table VII.

TABLE VII.—*Tests for proteases in the flesh of apples*

Material.	Amino-acid equivalent after action (milligrams of nitrogen).
Unboiled juice + egg albumin	0.12
Boiled juice + egg albumin	.07
Water + egg albumin	None.
Unboiled juice + peptone	.10
Boiled juice + peptone	.10
Water + peptone	.03

It appears from these data that both the juice itself and the peptone used contained amino acids which would give a blue color with the ninhydrin reagent. But the incubated mixture of unboiled juice and albumen contained more amino acids than that in which an equal volume of boiled juice was used; while with peptone no increase of amino acid was produced by the unboiled juice, and the total amino acid found was just equal to the sum of that found in the quantity of juice and of peptone solution used in the tests. It thus appears that the juice extracted by grinding with quartz sand contains a small amount of some protein-splitting enzym of the trypsin or papain type rather than of the erepsin type. It was concluded, therefore, that the flesh of the apples contains a small amount of protease, to the action of which on the protein material of the apple cells is due the small amount of amino acid found to be present in the juice of the ripening fruit.

PECTINASES

The fact that the flesh of an apple softens and becomes mealy or mushy at the close of the ripening period is generally attributed to the solution of the middle lamella and the consequent separation of the cells of the tissues. The solution of the middle lamella is supposed to be the work of an enzym known as pectinase. It is supposed, therefore, that pecti-

nase occurs in ripening fruits. It was intended at the outset to ascertain whether a pectinase was present in the apples used in this investigation, but review of the literature dealing with methods of detection of pectinase, as summarized by Cooley (4) in a recent article, together with the unsatisfactory results of Cooley's own use of these methods in testing for pectinase in diseased plums, made it appear doubtful that accurate evidence on this point could be secured. Some preliminary tests of the methods which had been suggested confirmed the writer's opinion in this respect, and the attempts were postponed until such time as more satisfactory methods of testing for pectinases have been devised.

ENZYMS IN THE SEEDS OF APPLES

Although the occurrence of different enzyms in the seeds of the apple would not have any bearing upon the ripening processes in the flesh of the apple and, hence, is of no importance to the particular object of this investigation, such an excellent opportunity was offered to test for enzyms in the seeds at the same time that the tests were being applied to the flesh or juice, that it was determined to carry on such a series of tests. Accordingly, a large number of seeds, some 20 gm. in all, were picked out of several apples, and the brown seed coat was picked from each seed. The white seeds were then kept for about two weeks in a vacuum desiccator until they were dry enough so that when crushed they gave off no odor of benzaldehyde, thus indicating that not enough water was present to permit the glucosidase action to occur.

A weighed quantity of the dry seeds was then ground in a mortar with sharp quartz sand until the seeds were thoroughly disintegrated. The material was then preserved in a tightly stoppered weighing bottle until needed for each test. For the tests, 2 gm. of the mixture, equivalent to 1 gm. of dry seeds, were digested at room temperature for 30 minutes with 100 c. c. of distilled water, and a filtered aliquot of this extract was used for the tests. A detailed description of the progress of each particular test is unnecessary in this article, but the results obtained, based upon a comparison of unboiled and boiled extracts with water controls, show the following facts with reference to the presence of the various enzyms which were tested for in apple seeds: Diastases, present in considerable amount; invertase, absent; emulsin, present in considerable amount; lipase, present in small amount; protease, present (hydrolyzes both albumin and peptone); oxidases, absent.

SUMMARY

From the results of these investigations it appears that the only enzyms which participate in the changes in the carbohydrates of apples during the ripening process are oxidases. None of the common types of carbohydrate-splitting enzyms could be found. The fact that the changes which take place during ripening are inhibited by surrounding the fruit in an atmosphere of carbon dioxid, as shown by the experiment described

in the opening paragraphs of this article, is easily explained on the basis of their being oxidase changes, since it is a well-known fact in enzymology that the presence of a large excess of the end products of a reaction generally inhibits the action of the accelerating enzym in increasing degree as the proportion of the end product increases. Carbon dioxid is undoubtedly the end product of oxidase activity and should therefore accomplish just the result which was found to occur in the jar in which this gas was used.

The small amounts of esterase and of protease which were found in the ripening apples indicate the possibility of the hydrolytic decomposition of the small quantity of essential oil and of protein material contained in the flesh of the apple during the ripening process or subsequent breaking down of the tissue.

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AN AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY FOR USE WITH FREELY EXPOSED PLANTS

By LYMAN J. BRIGGS, *Biophysicist in Charge, Biophysical Investigations*, and H. L. SHANTZ, *Plant Physiologist, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry.*

INTRODUCTION

An extended study of the transpiration rate of plants practically necessitates the use of an automatic balance of some type. The present paper contains a review of the various forms of transpiration balances heretofore employed, together with a description of a new automatic transpiration scale of large capacity, so designed that the plants may be freely exposed to the weather. Four of these scales have been in continuous use during the past four summers at Akron, Colo.

Automatic balances may be divided into two classes: (1) The step-by-step type, in which small weights of equal value are added to the scale pan in succession or a counterpoise is advanced in equal steps; (2) the continuous record type, in which the plant is suspended from a spring or from a variable lever or is mounted directly on a float.

RECORDING BALANCES OF THE STEP-BY-STEP TYPE

Vesque (1878)[1] appears to have been the first to employ an automatic balance in measuring transpiration. He made use of the step-by-step principle, a measured quantity of mercury being delivered to a receptacle on the scale pan each time the beam tipped sufficiently to close an electric circuit. His apparatus is illustrated in figure 1, the device for measuring the mercury being shown at *s* and enlarged at B. This measuring device is in principle similar to a large stopcock, in which the plug is only partially bored through from each side so as to form two shallow cavities of equal volume. Either cavity in its upper position becomes filled with mercury from the reservoir *t*. When the circuit is closed, a spring motor is released, which turns the plug through one-half a revolution, delivering the mercury in the cavity to the container *a*, and recording the time of the event by lowering the stylus *p* in contact with the circular plate *v* of the clock H.

Anderson (1894) was the first to employ steel balls of uniform size as weights for a recording balance. The balls were held in a spiral brass tube, with a block at the lower end containing a pocket for one ball. When the balance beam tipped sufficiently to close an electric circuit, the block was moved sidewise and the ball in the pocket dropped into the

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 131–132.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 3
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pan of the balance (fig. 2). The weight thus added opened the circuit, and a spring restored the block to its normal position, where the pocket was again filled by a ball from the reserve supply. Anderson did not

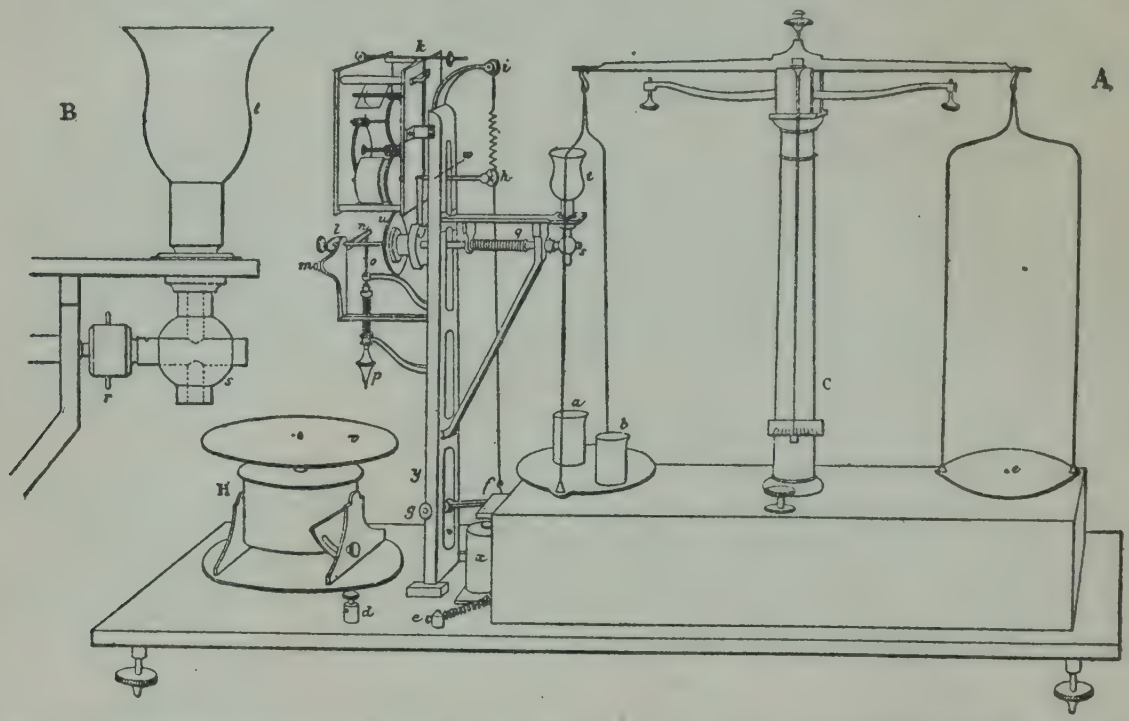

FIG. 1.—Vesque's automatic balance for measuring transpiration. In this apparatus measured quantities of mercury are added to the receiver on the balance pan to counterbalance the transpiration losses.

place the plant directly on the balance, but used his apparatus to register the gain in weight of absorption tubes connected with the transpiration chamber. He does not describe the form of the recording apparatus employed.

Ganong (1905) in his "autographic transpirometer" (fig. 3) combined the ball-dropping and the recording mechanism in a compact and con-

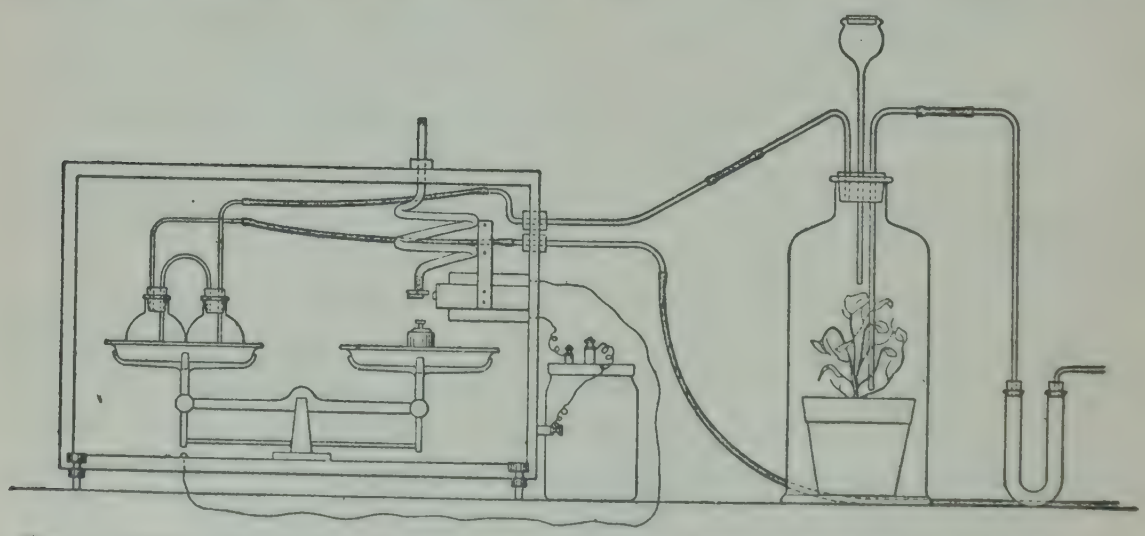

FIG. 2.—Anderson's apparatus for measuring transpiration, in which steel balls are used as weights.

venient form, one electromagnet serving both purposes. Steel balls one-fourth of an inch in diameter were employed as weights. Balls of this size approximate 1 gm. each in weight. The clock was so arranged

that by offsetting the cylinder daily a weekly record could be obtained on one sheet.

Transeau (1911), in working with xerophytes, employed hollow brass balls standardized to 0.4 gm. in place of ¼-inch steel balls of 1 gm. weight, but states that the hollow balls are not as light as could be desired. The writers have found that ⅛-inch steel balls weighing 0.13 gm. can be readily used, provided the valve [1] is constructed to fit them.

Woods (1895) used the automatic weighing rain gage of Marvin (1903) as a transpiration balance, the apparatus being modified to record loss instead of gain in weight (fig. 4). In this apparatus the counterpoise is

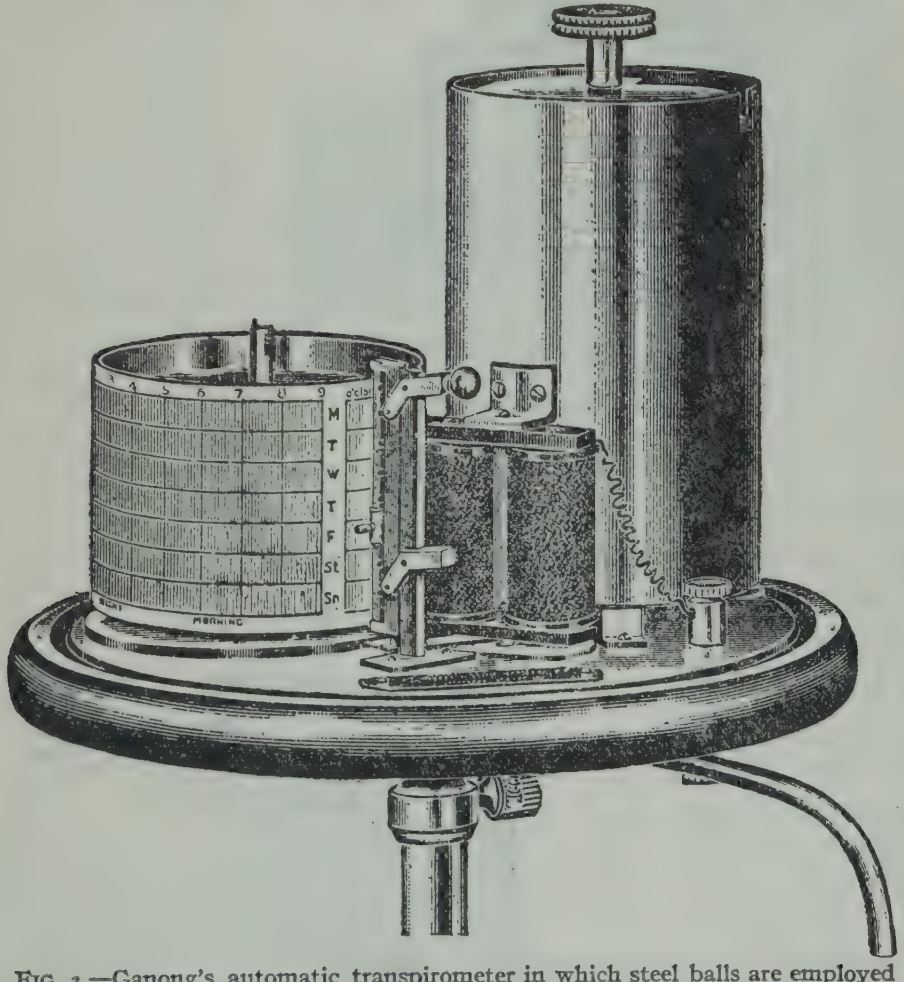

FIG. 3.—Ganong's automatic transpirometer in which steel balls are employed as weights.

moved along the beam in $\frac{1}{10}$-gm. steps by a screw actuated by an electromagnet carried on the balance itself. The recorder (fig. 5) is independent of the balance.

Blackman and Paine (1914) have recently described a recording transpirometer operating on the step-by-step principle, in which "water drops are used in place of steel balls, the water being added directly to the soil." Their apparatus has been represented schematically in figure 6. Water is allowed to drip continuously from a Mariotte system. During the greater part of the time the drops are intercepted by a movable

[1] For description of valve, see under "Ball-dropping device," p. 123.

funnel and collected as waste water. When the plant through transpiration causes the balance beam to tip sufficiently to close an electric circuit, the funnel F is withdrawn by the solenoid A, and the water drops fall directly into a receiving tube leading to the soil in the pot. Water is thus added directly to the pot until the balance tips sufficiently in the opposite direction to close a circuit through a second solenoid B, which restores the funnel to its intercepting position. The time at which the circuit is closed is electrically recorded on a clock drum. The position of the contacts is adjustable, so that the quantity of water added each time—i. e., the size of the steps—may be modified to suit the transpiration rate. This method is unique and advantageous in maintaining the moisture content of the soil constant throughout the experiment. Under freely exposed conditions, however, the quantity of water added each time would be variable and indeterminate, due to the oscillation of the balance by the wind.

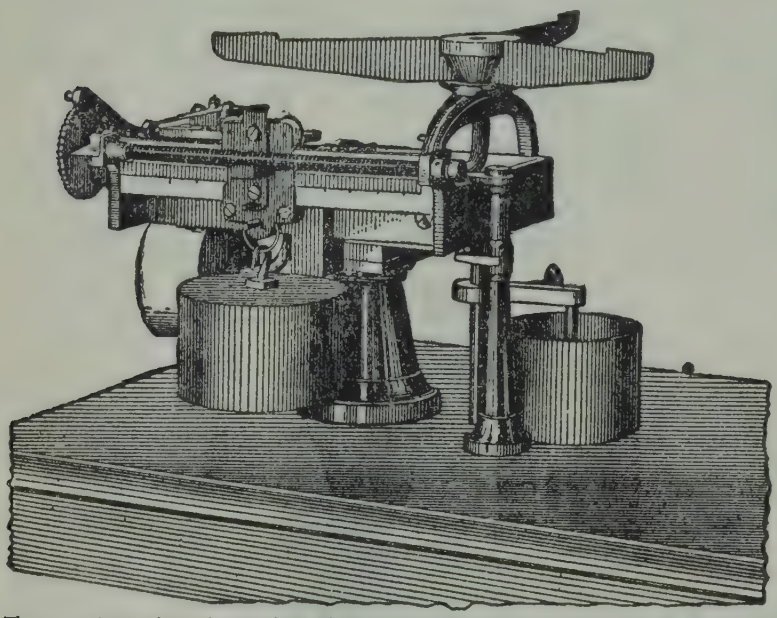

FIG. 4.—Woods' adaptation of Marvin's weighing rain gage as a transpiration balance. In this apparatus the loss through transpiration is counterbalanced by a weight controlled by a screw.

TRANSPIRATION BALANCES OF THE CONTINUOUS-RECORD TYPE

The first continuously recording transpiration apparatus appears to have been devised by Krutizky (1878). It is of interest to note that the first step-by-step recording apparatus was described by Vesque in the same year. Krutizky's apparatus is shown in figure 7. The water lost through transpiration from a potometer is continuously replaced through a siphon from a supply con-

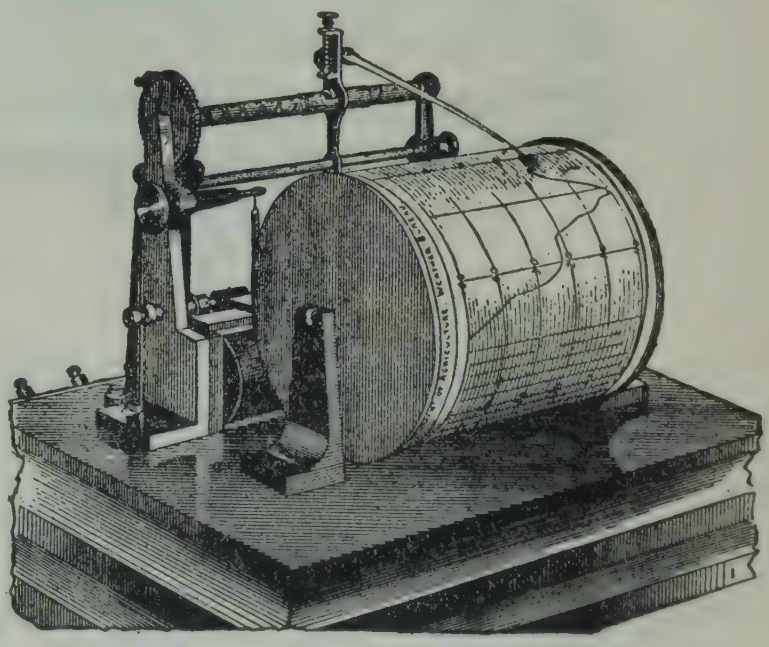

FIG. 5.—The Marvin register used by Woods for recording transpiration.

tained in a floating cylinder *a*, which rises as the load decreases and traces its movement on the smoked drum of a clock. Like other apparatus involving the principle of flotation, this apparatus is subject to errors arising from changes in buoyancy accompanying changes in temperature.

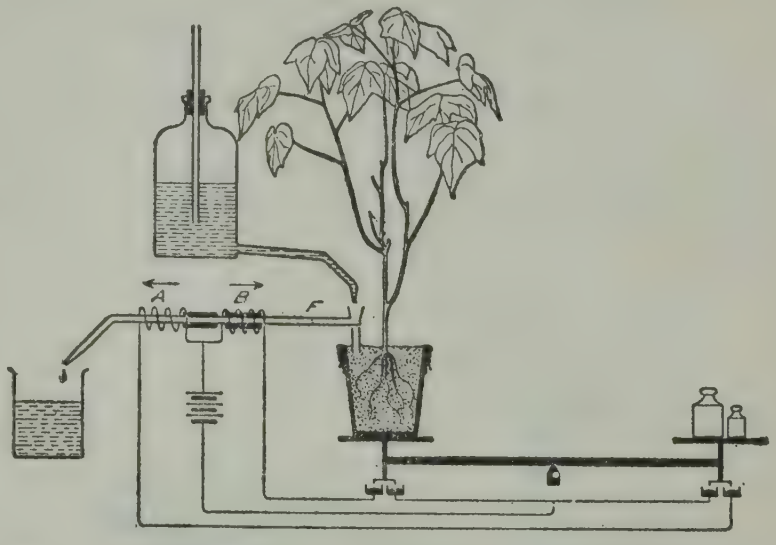

FIG. 6.—Schematic diagram of Blackman and Paine's recording transpirometer, in which water is automatically added to the pot to offset the transpiration loss, so that the moisture content of the soil is kept uniform.

A transpiration balance devised by Richard Frères (Burgerstein, 1904, p. 8–9) is illustrated in figure 8. The balance is made very insensitive by a heavy bob. The movement of the balance pan from the "down" to the "up" position corresponds to a known loss in weight, depending on the weight and position of the bob. The movement of the beam is recorded directly on the drum of a clock.

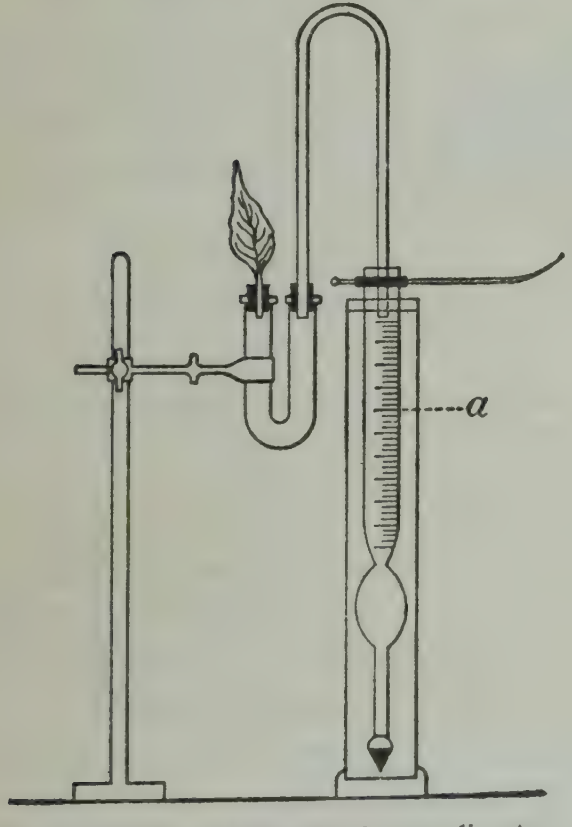

FIG. 7.—Krutizky's potometer for recording transpiration, in which the loss from the potometer is continuously replaced from the supply in the floating cylinder.

Copeland (1898) has described an apparatus (fig. 9) for recording transpiration in which the weight of the plant is balanced over a pulley by the weight of a partially submerged hydrometer bulb. The pulley shaft rolls on plate-glass supports to reduce the friction. A tracer supported from a second wheel records the motion on smoked paper on a clock cylinder. With its maximum load (3.5 kgm.) the instrument responds to a change in weight of 0.05 gm.

Corbett (1900) has used a large Nicholson hydrometer for measuring transpiration, the plant being placed directly on the pan *a* of the hydrometer *b* (fig. 10). The apparatus is made self-recording by connecting the float with the lever of an auxanometer. This apparatus, like that of Copeland, is affected by temperature, which changes the density of the water and consequently

its buoyancy. Temperature effects can, however, be practically eliminated by surrounding the hydrometer tank with a water-jacket, through which water is constantly circulating. The sensibility of the apparatus is determined by the cross section of the stem of the hydrometer.

A NEW AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY

The requirements of the transpiration studies at Akron necessitated an automatic weighing apparatus having a carrying capacity of 150 kgm., capable of operating positively in the wind, and so designed that the plants could be freely and continuously exposed to the weather (Pl. IX). A platform scale with agate bearings having a carrying capacity of 200 kgm. and a sensibility of 5 gm. was chosen for equipment as an automatic balance of the step-by-step type (Pl. X). The scale was fitted with a short column so as to bring all the mechanism below the level of the top of the pot and was provided with the following auxiliary equipment:

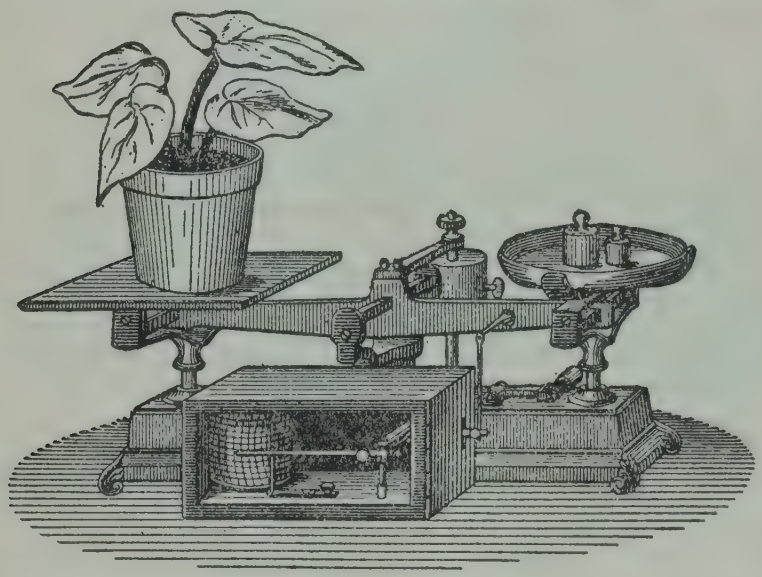

FIG. 8.—The transpiration balance of Richard Frères with its recording apparatus.

a. Ball-dropping device.
b. Ball receiver on beam.
c. Beam contact and mercury cups.
d. Oil dashpot on beam.
e. Spring motor for raising beam.
f. Adjustable counterpoise for raising the center of gravity of balanced system.
g. Recorder for registering time at which each ball is dropped.
h. Batteries and relay.
i. Case for protecting mechanism from the weather.

The beam of the scale with a part of the auxiliary equipment is shown in fig. 11. The operation of the mechanism is briefly as follows: As the plant decreases in weight, the beam falls until an electric contact is made at U. This closes a relay circuit, with the following results:

1. The ball-dropping device A deposits a ball in the receiver L. The weight of this ball tends to raise the beam.
2. The spring motor, by means of a cam K, raises the beam promptly and positively to its upper position.
3. The time of the event is indicated on the drum of the recorder.

BALL-DROPPING DEVICE.—The ball-dropping device used in our experiments is shown in fig. 12. A commercial telegraph sounder provides an efficient mechanism for actuating the valve. When the circuit is closed, the slide A moves in the direction of the arrow and releases the lowest ball in the tube. The remaining balls are prevented from passing down the tube by the upper septum B, which moves into the tube as the lower septum C moves out. When the circuit is broken, a spring restores the valve mechanism to its original position and the reserve balls slide down the tube so as to rest against the lower septum. The mechanism is now in position to drop another ball as soon as the circuit is again closed.

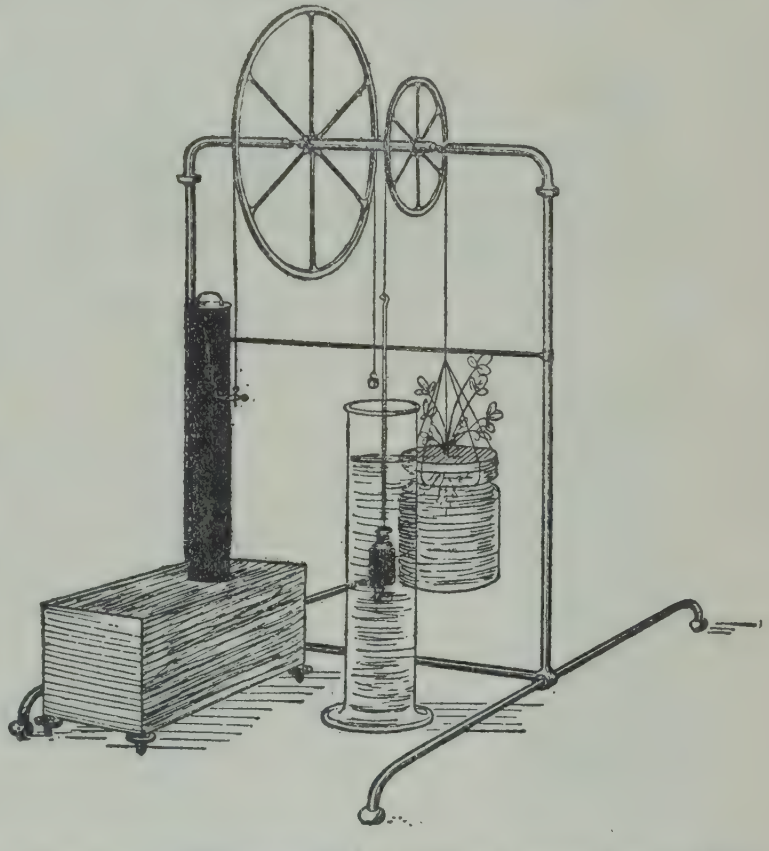

FIG. 9.—Copeland's apparatus for recording transpiration in which the loss in weight through transpiration is counterbalanced by a change in position of a partially submerged float.

As the discharged ball leaves the valve it drops into the balanced receptacle D, which tips downward under the weight of the ball, closing the circuit of the recorder through the mercury cups E below. The ball in the meantime rolls into the funnel and is delivered into the ball receiver L, suspended from the balance beam. With this arrangement no record is made unless the ball is actually received in D, and a second ball can not be recorded until the first has been delivered and D has returned to its initial position. In very gusty weather there is occasionally a fluttering of the valve A, two balls being dropped in rapid succession. The second ball simply shoots over D into the waste cup and is not recorded.

The tube holding the reserve supply of balls (fig. 11) is of glass bent into the form of an open spiral, and is joined to the valve tube by a conical adapter. The diameter of the valve tube at the septa must be only slightly greater than the diameter of the balls to insure the valve's working properly, and the tube should taper gradually to this diameter. The distance between the adjacent faces of the two septa should also be equal to the diameter of the ball. Each septum when in its intercepting

position should extend into the tube approximately one-fourth of the tube diameter. It is essential that the valve be accurately made to conform to the particular size of ball used as a weight. The inside of the valve tube should be kept smooth and clean by the occasional use of benzine, and the balls should also be kept polished.

The balls used for weights were three-sixteenths of an inch in diameter and of first-quality hardened steel. They were found to be so nearly uniform in weight that no appreciable error is introduced by assuming them equal. The individual weights in milligrams of 10 balls selected at random were as follows: 437.0, 438.5, 437.2, 437.7, 436.8, 437.6, 437.3, 438.0, 437.5, 437.0. Mean, 437.4. Probable error for a single ball, 0.4 mgm., or 1 part in 1,000.

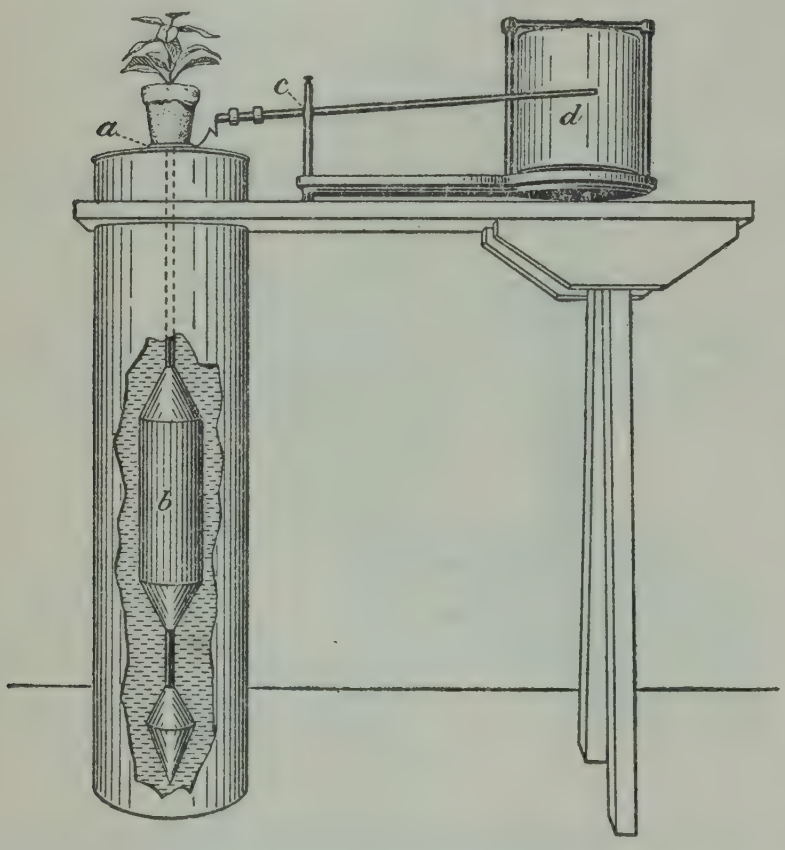

FIG. 10.—Corbett's apparatus for measuring transpiration in which the plant is carried on the pan of a large Nicholson hydrometer.

BALL RECEIVER.—The conical receiver L for the balls is suspended from an extension of the beam (fig. 11) on the same side as the load, since the added weight of the ball compensates for the loss by transpiration. The receiver is suspended from a knife-edge which lies in the plane determined by the two other knife-edges on the beam. The distance from the central knife-edge is so chosen that the weight of a ball corresponds to a change of 20 gm. in the weight on the scale platform.

The measuring tray shown in Plate XI affords a rapid means of counting the balls dropped during any period without touching them. Each complete row includes 10 balls, and the rows are graduated accordingly on the margin. It is essential that the lower end of the tray be cut obliquely so as to form an angle of 60° with the graduated side.

DASHPOT.—The oil dashpot (fig. 13) is an essential part of the apparatus when the balance is exposed to the wind. The resistance can be adjusted to some extent by turning the perforated plate on the top of the inner cylinder I. The outer cylinder O is mounted directly below the weight support on the beam, to which the inner cylinder is attached by the rod N. (See fig. 11.)

SPRING MOTOR FOR RAISING BEAM.—The dropping of a ball into the receiver is ordinarily sufficient to raise the opposite end of the beam and open the circuit. It sometimes happens, however, when the transpiration rate is high and a gusty wind is blowing, that the beam remains down until the transpiration has been sufficient to require a second ball

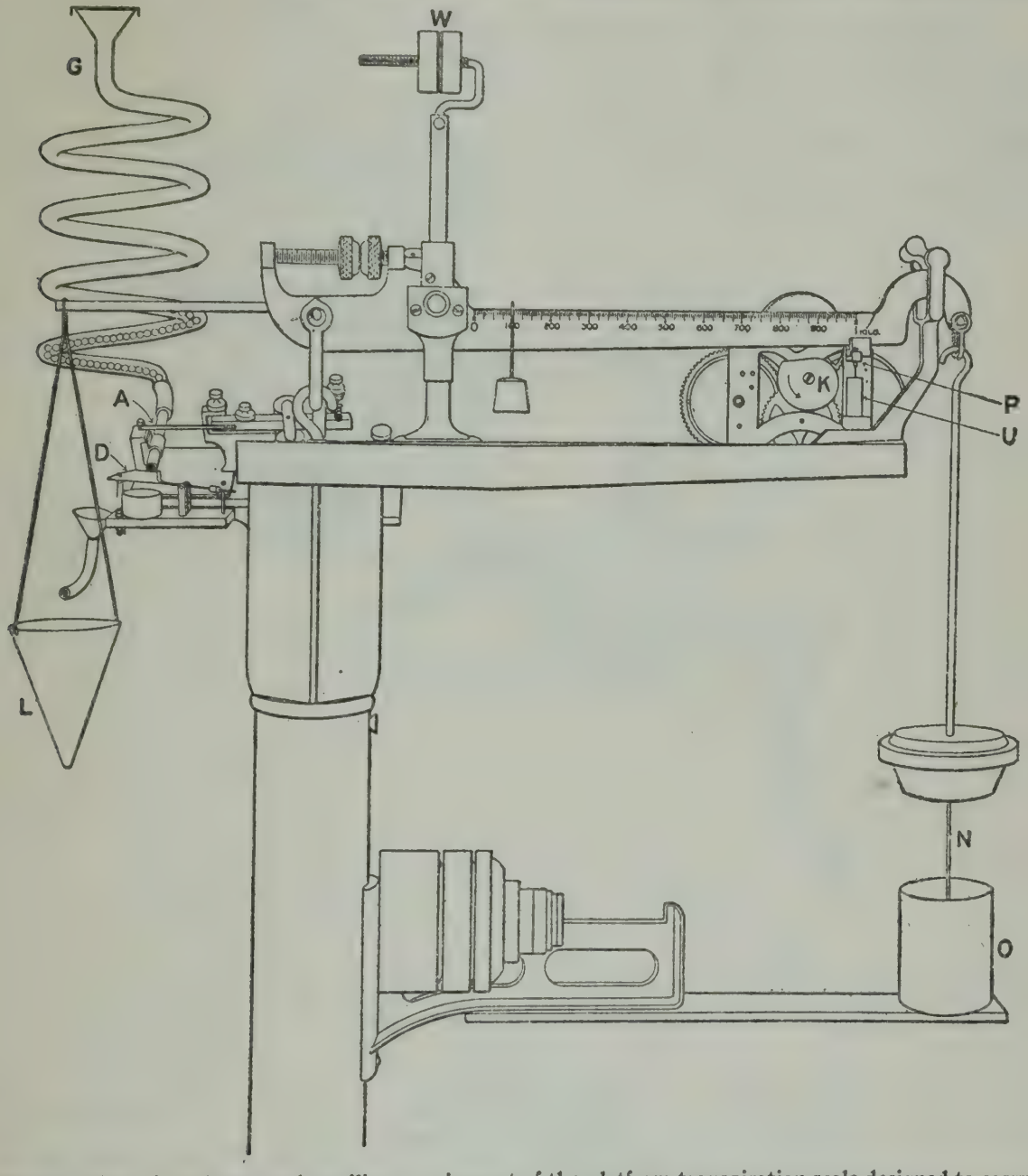

FIG. 11.—View of the beam and auxiliary equipment of the platform transpiration scale designed to carry large pots of plants weighing 150 kgm. or more. As the plant loses weight, the beam falls and the platinum point P closes a circuit through the mercury cup U. This actuates the ball dropper A, which deposits a ball in the receiver L. At the same time the cam K makes one revolution, raising the beam to its upper position and leaving it free to fall. An oil dashpot is provided at O.

to counterbalance the loss in weight. Under such conditions the balance would fail to operate without the intervention of some protective device. This protection is secured by a spring motor which raises the beam to its upper position each time a ball is dropped and then leaves the beam free. The motor, which consists of a strong 8-day clock movement equipped with a fan, F (fig. 14), to reduce the speed, is controlled by

an electromagnet, M (fig. 15). When the beam circuit is closed, the motor is released and raises the beam through a cam, K (fig. 14). When the cam shaft S (fig. 15) has completed one revolution, the arm H on the cam shaft again engages the spring R on the armature T of the magnet, and the motor is stopped.

ADJUSTABLE POISE FOR RAISING CENTER OF GRAVITY OF BEAM.—It is essential that the mercury contact on the beam be closed with a positive motion to avoid the fluttering of the relay armature. This is accomplished by raising the center of gravity until the beam is slightly unstable, by means of an adjustable bob, W, located above the central knife-edge. (See fig. 11.)

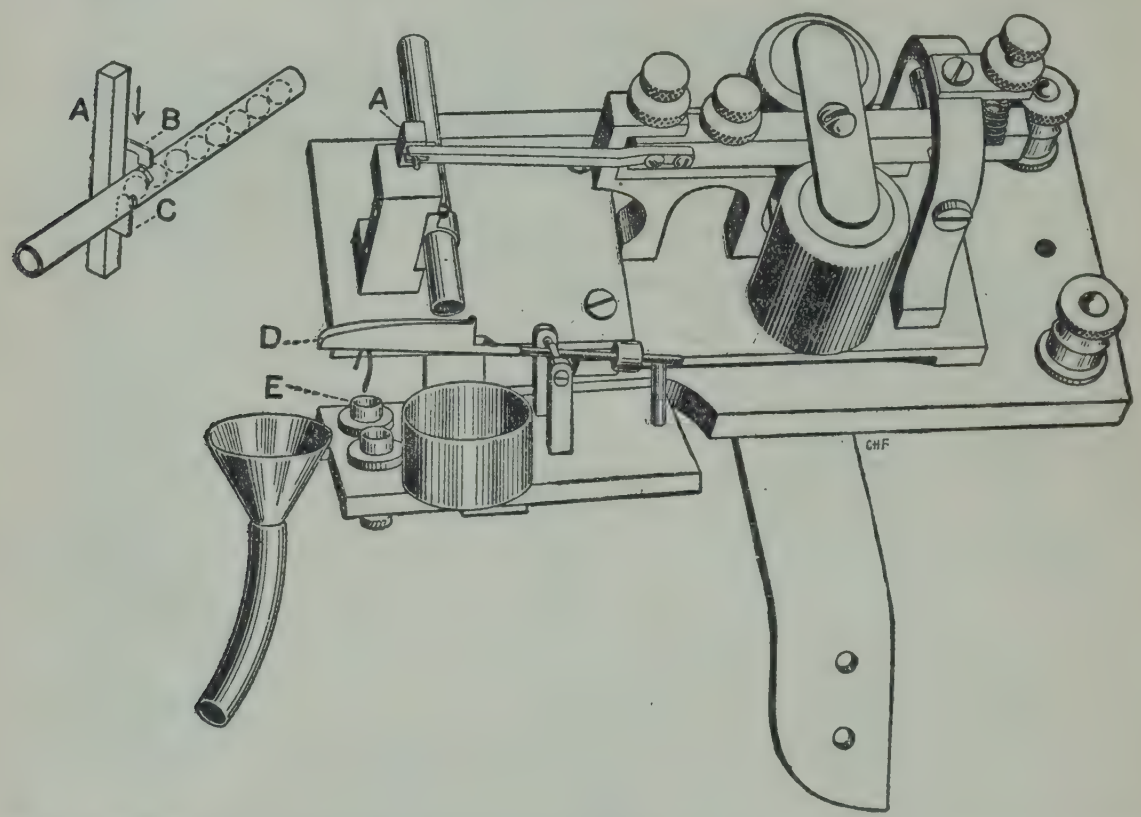

FIG. 12.—Details of the ball-dropping mechanism. The steel ball passes through the valve A into the tipping bucket D, which falls under the weight of the ball and closes an electrical circuit at E to the register.

MARVIN RECORDER.—A convenient type of recorder for registering the time at which each ball is delivered is that devised by Marvin for use in connection with automatic rain gages. This recorder has a drum, 12 inches in circumference, which makes one revolution in six hours and is continously offset by a screw, so that the four 6-hour periods are recorded side by side on the same sheet. A valuable feature is a zigzag attachment on the magnet, by means of which the tracing pen is permanently displaced each time the magnet circuit is closed. This gives a record which is much easier to read than the ordinary record in which the pen returns to its initial position when the circuit is opened (fig. 16). The dropping of two balls in rapid succession is easily seen in the zigzag

record on account of the double offset, but is difficult to determine in a record of the ordinary type.

PROTECTING CASE.—A tight weatherproof case inclosing the column and beam of the balance protects the automatic equipment from the

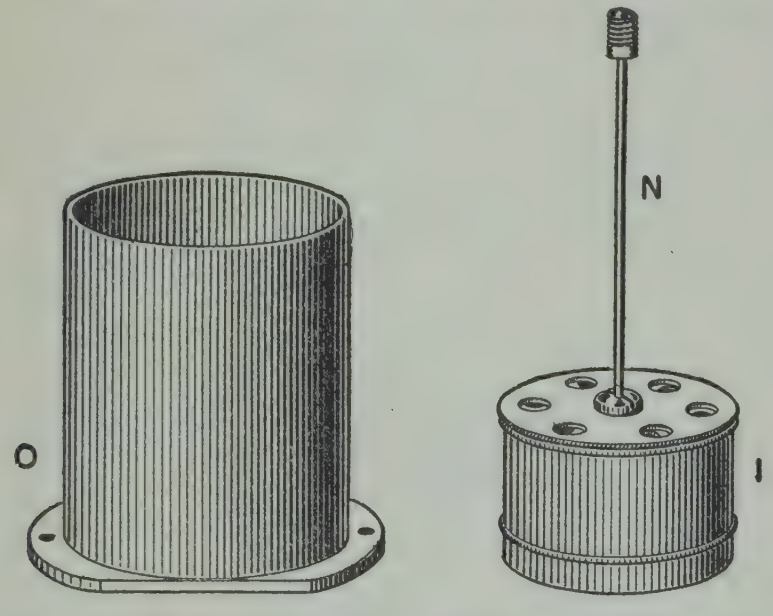

FIG. 13.—Dashpot for preventing the oscillation of the beam during windy weather.

weather. The case is equipped with a removable top and a sliding front. The latter is also supplied with a smaller door through which the apparatus can be observed and adjusted.

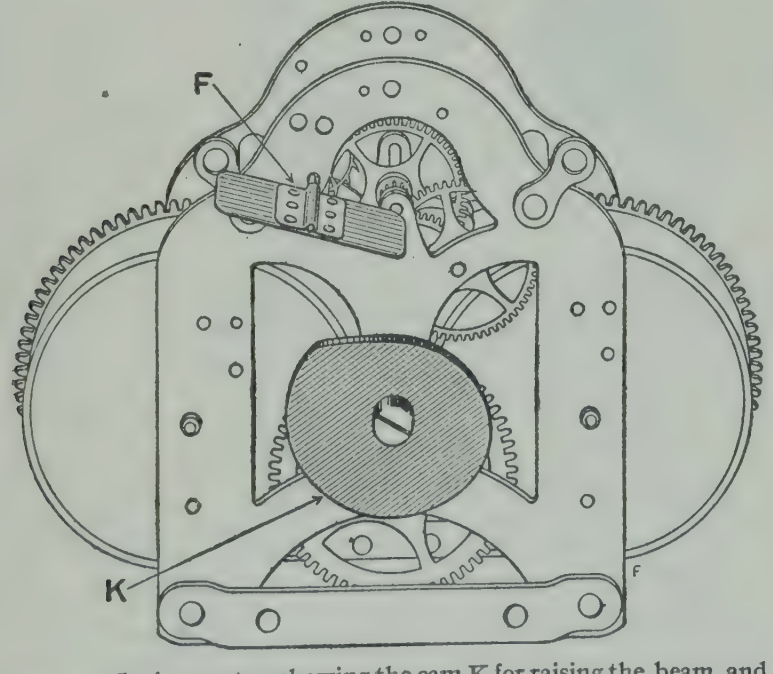

FIG. 14.—Spring motor, showing the cam K for raising the beam, and the fan F for regulating the speed.

ELECTRIC CIRCUITS.—The electrical connections consist of three circuits (fig. 17). A single dry cell, B_1, operates the relay through the beam contact. The ball valve and the motor release are connected in parallel in a second circuit, B_2, containing a battery of three or four cells. This

circuit is controlled by the relay contact. The recorder is operated by a third circuit, B_3, controlled by the tipping bucket on the ball valve. Each circuit is closed only momentarily, and the dry cells usually need to be renewed but once during the summer.

AUTOGRAPHIC RECORDS FROM THE AUTOMATIC TRANSPIRATION SCALE

The results of our transpiration measurements will be presented in other papers, but it seems desirable to reproduce here several daily records illustrating the actual performance of the apparatus. A word of explanation in connection with the interpretation of the records may be helpful. The clock drum makes four revolutions during the day, so that the record is divided into four 6-hour periods. The pen is offset at the moment each ball is delivered. There are five such offsets or steps in one direction (up, for instance) and then five steps in the opposite direction. Since each offset corresponds to a loss of 20 gm. of water, the interval from the crest to the trough of the graph is the time required for the transpiration of 100 gm. of water, or from crest to crest, the time interval for 200 gm. loss.

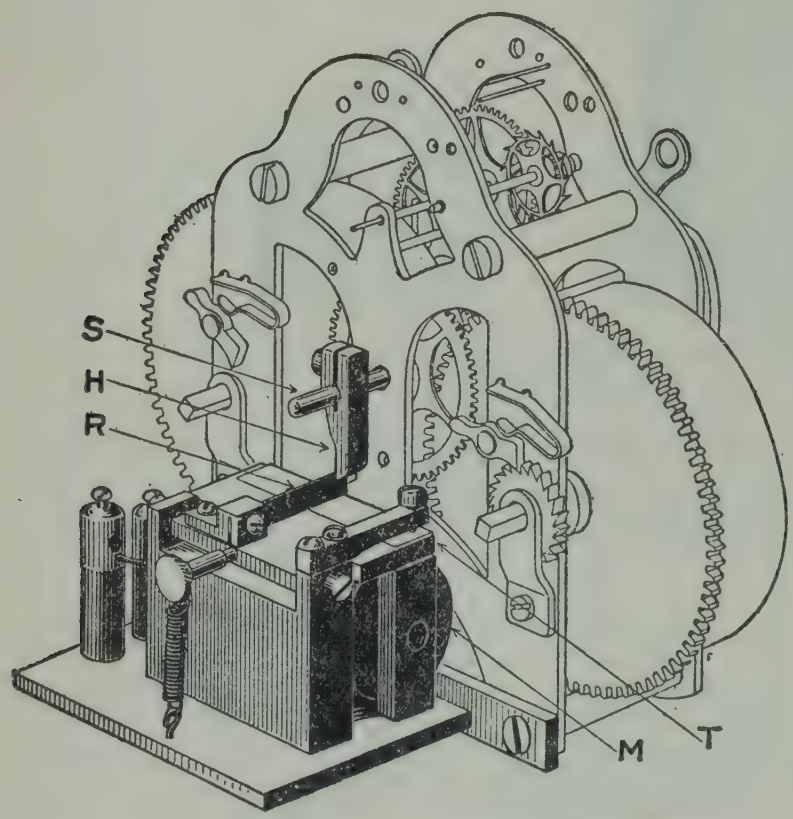

FIG. 15.—Another view of the spring motor, showing the control mechanism. When the magnet M is energized, the spring R attached to the armature T is pulled down, releasing the motor. Raising the beam de-energizes M, so that the motor, after making one revolution, is stopped by H again coming in contact with R.

The wheat records shown in figure 16 were taken from a series obtained in 1912 inside the screened inclosure used in the water-requirement experiments. The normal wind velocity was reduced somewhat by the inclosure and by the proximity of other plants. The first record reproduced (July 2, 1912) was obtained on a clear day. It will be noted that the time interval shortens as midday is approached—that is, the transpiration rate increases and attains its maximum value about 3 p. m., after which it falls rapidly. The transpiration at night, represented by

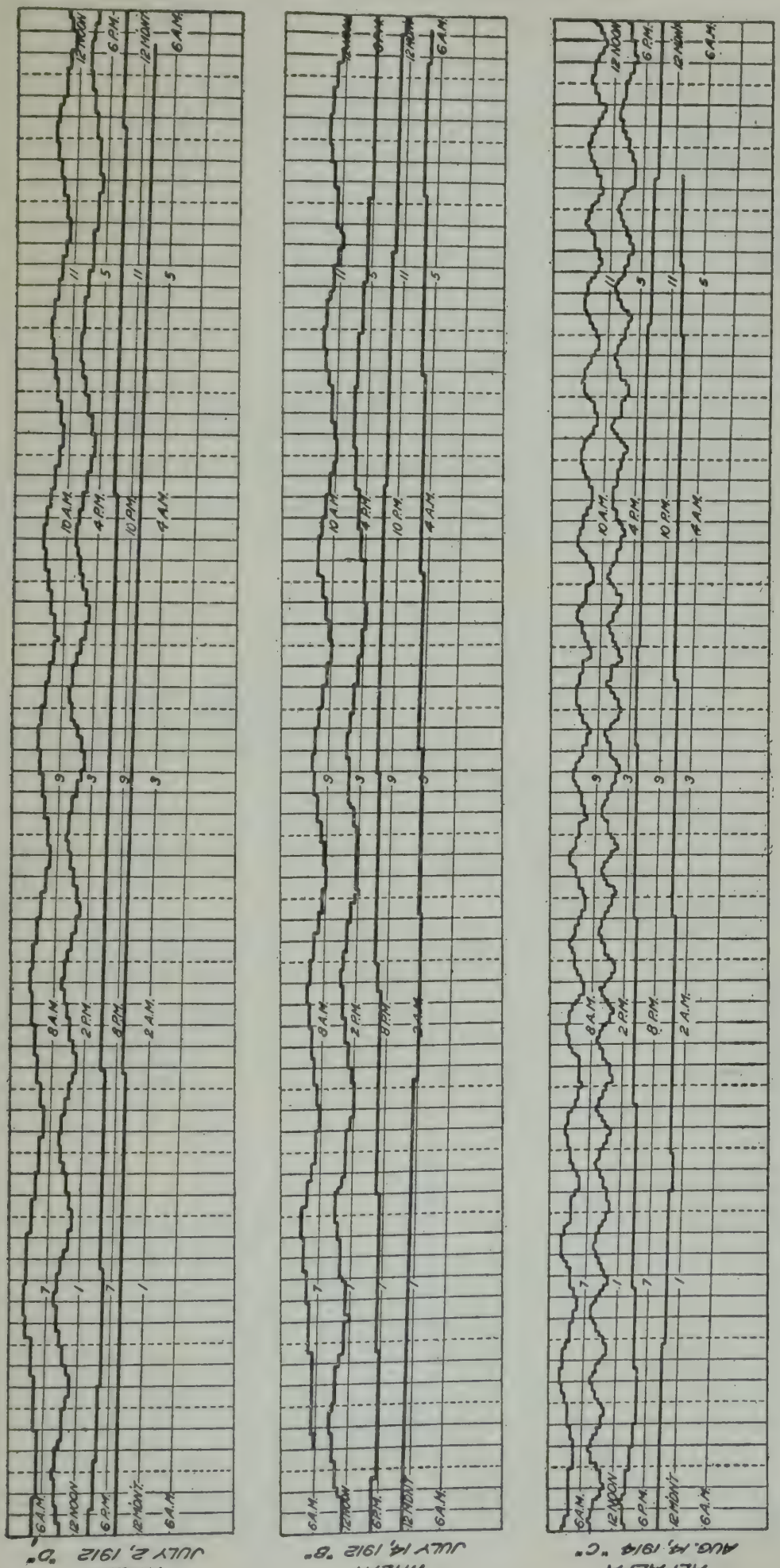

FIG. 16.—Sample records from the automatic transpiration scale. Each step corresponds to a transpiration loss of 20 gm., or 100 gm. from crest to trough of the graph.

the two lower lines of the graph, is seen to be very small as compared with the day transpiration.

The second graph for wheat (July 14, 1912) was selected to show the effect of cloudiness in the afternoon, beginning at 3.30 p. m. The change in the transpiration rate is seen to occur soon after this, and the transpiration between 5 and 6 p. m. is very low compared with that on a clear day, as shown by the first chart. The transpiration during the night of July 14 was higher than during the night of July 2. Automatic measurements with a wet-bulb instrument show that the air contained less moisture during the night of July 14 than during the night of July 2, which would account for the increased transpiration. The temperature on the two days was practically the same.

The third chart shows a record of a pot of alfalfa, taken outside the inclosure. The plants were freely exposed to the wind, which ranged in velocity from 7 to 14 miles per hour during the morning and from 2 to 5 miles during the afternoon. Over 8 liters of water were transpired

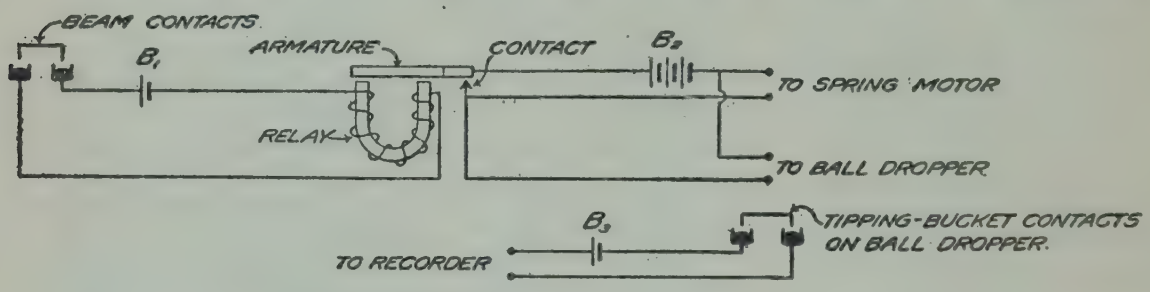

FIG. 17.—Wiring diagram of the electric circuits of the automatic transpiration scale.

during the day, and it is of interest to note how closely this loss is confined to the daylight hours.

The transpiration recorded on the three record sheets reproduced in figure 16 is plotted in rectangular coordinates in figure 18, showing for each pot of plants the transpiration rate in grams per hour for each hour of the day. It may be added that the pots used were equipped with sealed covers, so that the loss of water by direct evaporation from the soil was practically eliminated.

SUMMARY

This paper describes an automatic transpiration scale of 200 kgm. capacity and 5 gm. sensibility, designed for use in connection with the large culture pots employed by the writers in water-requirement measurements. The apparatus is so constructed that the plants may be freely exposed to wind and weather. Steel balls are used as weights, as in Anderson's balance, each ball corresponding to a change in weight of 20 gm. A spring motor is provided to lift the beam positively when a ball is dropped, which is an essential feature when plants are exposed to wind. The apparatus works very satisfactorily except in the presence of whirlwinds or sudden gusts, which lift the plants and tend to give a transpiration

rate which is momentarily too high. Special provision is made to prevent two balls being delivered to the beam in rapid succession, and no record is made unless a ball is actually delivered to the ball container on the beam. Four of these automatic scales have been in use during the past four summers at Akron, Colo., and continuous records have been secured during these periods. The results of these measurements will be discussed in other papers.

A brief review is also given of other forms of transpiration balances, which are divided into two classes: Those operating on the step-by-step principle, which includes the balances here described, and those of the continuous-record type. The first class includes balances in which the adjustment is secured by adding small weights (solid or liquid) of equal mass or by moving a counterpoise in uniform steps. In the second class the plant is suspended from a spring, or from a variable lever, or is mounted (directly or indirectly) on a float.

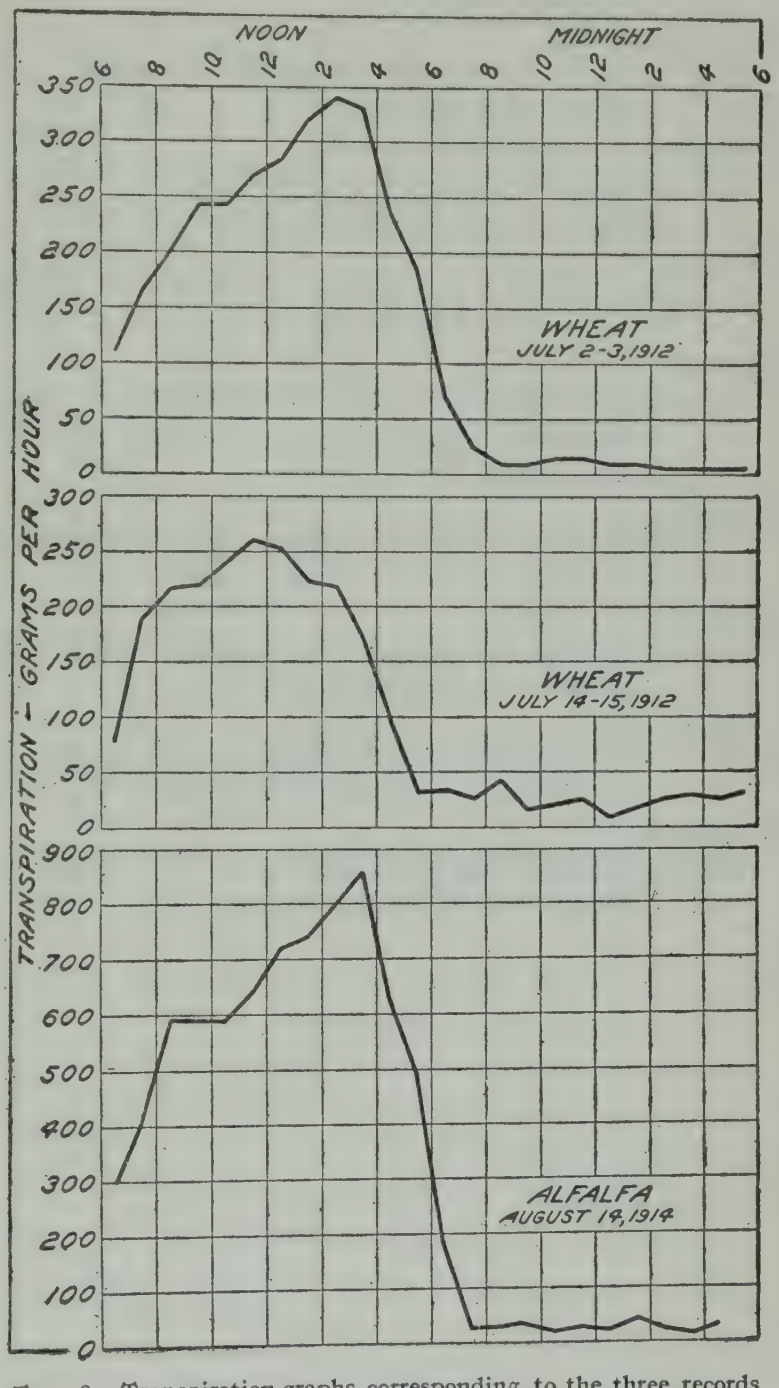

FIG. 18.—Transpiration graphs corresponding to the three records given in figure 16, plotted in rectangular coordinates.

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PLATE IX

Fig. 1.—Four automatic balances in operation at Akron, Colo., June 19, 1912, with the front of the box containing the mechanism open. The recording device is shown just beyond the first box. A separate recorder is used for each instrument.

Fig. 2.—Automatic balances, Akron, Colo., July 24, 1912; boxes closed and recorders covered. Except when being adjusted, this is the condition in which the apparatus is maintained.

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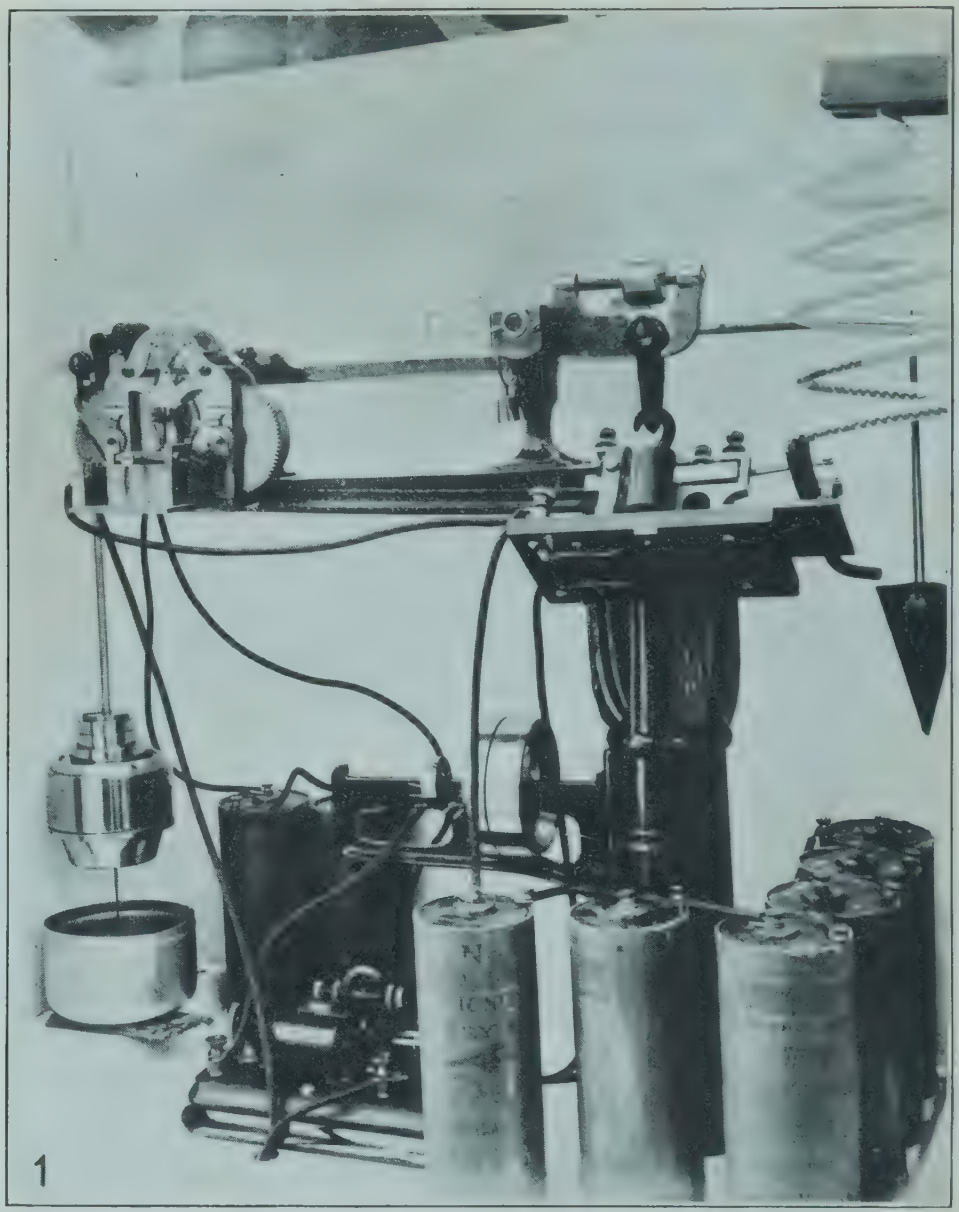

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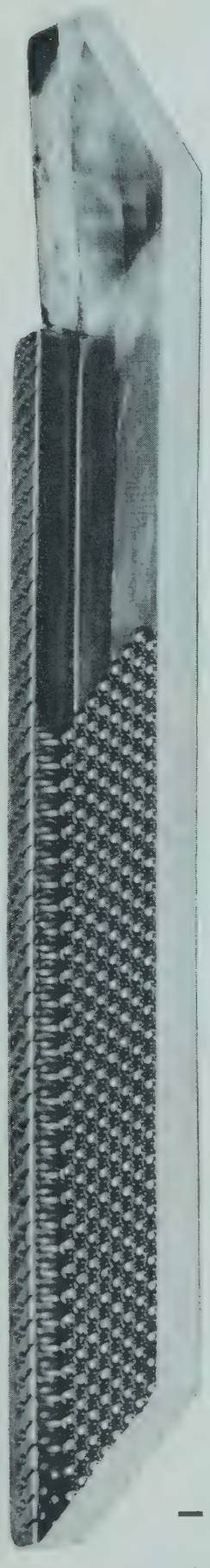

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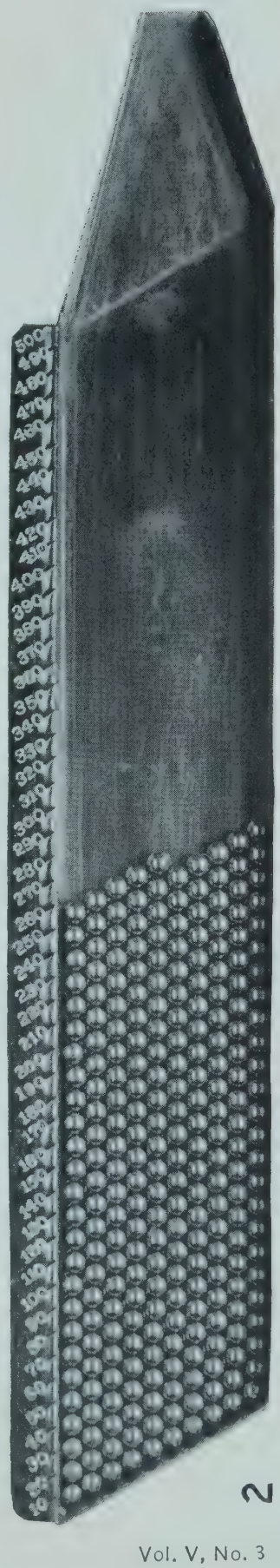

2

PARASITISM OF COMANDRA UMBELLATA

By GEORGE GRANT HEDGCOCK,
Pathologist, Investigations in Forest Pathology,
Bureau of Plant Industry

One of the most important and most injurious of the stem or blister rusts occurring on pines is *Peridermium pyriforme* Peck, which attacks *Pinus* (*murrayana*) *contorta* Loud., *P. ponderosa* Laws., and *P. ponderosa scopulorum* Engelm. in the western United States, *P. divaricata* Du Mont de Cours. in the Northern States, and *P. pungens* Michx. and *P. rigida* Mill. in the Northwestern States. *Peridermium pyriforme* is a heterœcious rust and is dependent for its existence upon its alternate, or summer, stage, which occurs on species of Comandra.

The problem of the eradication of this important rust being so intimately associated with plants of *Comandra* spp. led the writer to investigate their manner of growth and means of propagation. It was found that the plants of at least two species, *C. pallida* A. DC. and *C. umbellata* (L.) Nutt., have apparently become largely dependent on parasitism for their continued existence. The other two North American species, *C. livida* Richards, and *C. richardsiana* Fernald, resemble the former species in appearance and habit and are probably equally parasitic in their nature.

The writer has carefully examined the root system of living plants of both *C. umbellata* and *C. pallida*, but only of dried specimens of the other two species. The former have long underground rootstocks which bear here and there small roots or rootlets usually less than 5 inches in length. These rootlets branch sparsely and are nearly always attached to the roots or underground stems of other species of plants. At the point of attachment there is formed by the root of *Comandra* spp. a nearly hemispherical disk or holdfast. This holdfast is either superficial or slightly embedded in the cambium layer of tissues of the host, but does not send out haustoria, as is the case in species of Razoumofskya on the limbs and trunks of coniferous trees. The chief function of the roots of *Comandra* spp. appears to be that of attachment to host plants for the purpose of obtaining nourishment and a water supply. Plants of *Comandra* spp. frequent dry, rocky soils, which often have a low water content.

Plants of all these species of Comandra bear leaves; and although attached as parasites to the roots of other plants, they are not entirely dependent upon their host plants for organic compounds, since they are able to further elaborate these compounds in the liquids received from

Journal of Aricultural Rsearch.
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Vol. V, No. 3
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their hosts. In this respect their development is similar to that of plants of species of Phoradendron.

Both *C. umbellata* and *C. pallida* very commonly are associated with and parasitic upon species of Vaccinium, but are not at all dependent upon this genus for host plants. This has especially been noted in the case of *C. pallida* in the States of Colorado, Montana, Nebraska, South Dakota, and Wyoming, and in *C. umbellata* in the States of Connecticut, Maryland, Michigan, Minnesota, New Jersey, New York, Pennsylvania, Vermont, Virginia, and Wisconsin, and the District of Columbia. Plants of both species are parasitic upon a great variety of plants belonging to widely different sections of the Spermatophyta. No attachment to plants of any member of the Pteridophyta has been noted.

C. umbellata has been found by the writer as a parasite on the roots of the following species of plants in the Eastern States:

Acer rubrum L.
Achillea millefolium L.
Andropogon virginicus L.
Angelica villosa (Walt.) B. S. P.
Antennaria plantaginifolia (L.) Richards.
Aster ericoides L.
Aster macrophyllus L.
Aster patens Ait.
Aster undulatus L.
Baptisia tinctoria (L.) Br.
Betula nigra L.
Betula populifolia Marsh.
Carex sp.
Castanea dentata (Marsh.) Borkh.
Chimaphila umbellata (L.) Nutt.
Chrysopsis mariana (L.) Nutt.
Comptonia peregrina (L.) Coulter.
Danthonia compressa Austin.
Fragaria americana (Porter) Britton.
Fragaria virginiana Duchesne.
Gaylussacia frondosa (L.) T. and G.
Hieracium venosum L.
Ionactis linariifolius (L.) Greene.
Lespedeza violacea (L.) Pers.
Lysimachia quadrifolia L.
Meibomia paniculata (L.) Kuntze.
Panicum sp.
Poa compressa L.
Poa pratensis L.
Populus tremuloides Michx.
Potentilla monspeliensis L.
Quercus coccinea Muenchh.
Quercus digitata (Marsh.) Sudw.
Quercus marilandica Muenchh.
Quercus nana (Wood) Britton.
Rhus copallina L.
Rosa blanda Ait.
Rosa canina L.
Rubus canadensis L.
Rubus procumbens Muhl.
Rubus villosus Ait.
Solidago bicolor L.
Solidago caesia L.
Solidago juncea Ait.
Solidago nemoralis Ait.
Solidago speciosa Nutt.
Spiraea salicifolia L.
Vaccinium atrococcum (A. Gray) Heller.
Vaccinium nigrum (Wood) Britton.
Vaccinium vacillans Kahn.

In addition to the foregoing and incomplete list there must be added at least three unidentified species of grasses.

During the last three years a number of attempts, with varying success, have been made at Washington, D. C., to grow plants of *C. umbellata* and *C. pallida*, both by germinating the seed and by transplanting rootstocks to beds and pots in greenhouses. In every case where living rootstocks unattached to host plants have been transplanted to pots or

beds without the host plants present, little or no growth on the part of the plants of *Comandra* spp. has taken place, and the plants eventually died. Successful results in growing these species have been accomplished by only two methods: First, by transplanting sods containing the plants of *Comandra* spp. from out of doors to the greenhouse without breaking the attachments of the roots of the parasite to those of the host; second, by planting seed in flats in the fall out of doors and germinating them in the presence of the roots of host plants after exposing the seeds to freezing temperatures by allowing the flats to remain out of doors all winter.

Dr. E. P. Meinecke, of the Office of Forest Pathology, reports by letter that he has three plants of *C. umbellata* raised from seed sown in 1913, which remained dormant till 1915, when they germinated and grew without any host plant. These plants were 5 inches high on July 17, 1915. This is positive proof that this species of Comandra can live without parasitism if necessary. It remains to be seen whether these plants will continue to grow indefinitely without the presence of host plants.

The results from our experiments indicate that when the rootstocks of plants of *Comandra* spp. are broken entirely loose from their root attachment to host plants they usually die through an inability to reattach themselves. These new data on a subject which apparently has not been previously investigated indicate a greater degree of parasitism in species of Comandra than has hitherto been suspected, and will render more obvious the desirability of the destruction of plants of *Comandra* spp. in the vicinity of forest-tree nurseries.

SEPARATION OF SOIL PROTOZOA[1]

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Some interesting problems have been suggested by the contention of Russell and Hutchinson (9, 10)[2] that protozoa are one of the limiting factors in soil fertility, because they feed upon and consequently limit the numbers of soil bacteria. Before the agricultural scientist can successfully formulate a complete explanation of the phenomena concerned with the function of protozoa in soils it is essential to establish certain fundamentals in methodology. Russell and Hutchinson (9, 10) and Cunningham (2, 3) have presented some valuable information concerning the depression of bacterial numbers as a result of inoculation with cultures of protozoa. The writers entered upon an investigation of a similar nature, with an attempt to base their work upon the use of protozoa-free cultures of bacteria, and bacteria-free cultures of protozoa.

But little mention is to be found in the literature regarding the separation of the different kinds of protozoa from each other and from bacteria. Russell and Hutchinson (9, 10) and Fred (4) have employed an efficient method of filtration for obtaining cultures of protozoa, but they do not offer any further experimental data concerning such separations. Cunningham (2, 3) has made use of a single-drop method for obtaining protozoa-free cultures of bacteria, based on the transfer to a suitable medium of a drop from a protozoan culture which upon microscopic examination revealed no protozoa. On the other hand, he does not describe any direct method for obtaining a bacteria-free culture of protozoa. Jordan (5, p. 469) mentions a method which might prove somewhat tedious—that is, having protozoa pass through concentric rings of dead bacteria on a culture plate until they had no living adhering bacteria. He refers also to Frosch's[3] method of separation by means of a sodium-carbonate solution. Richter (8) suggests the use of a high-gelatin medium which would suppress the bacterial growth of liquefying organisms. Biffi and Razzeto (1) give an account of the passage of protozoa through semipermeable filters after a considerable period of time has elapsed.

The writers are in agreement with Biffi and Razzeto regarding the importance of the time element in filtration, since it has been observed that protozoa have been able to work through the pores of a filter in a short time.

In the work under consideration—namely, the separation of flagellates from ciliates—an 8-day-old culture of soil organisms was employed.

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[1] From the Departments of Soil Chemistry and Bacteriology, New Jersey Experiment Station, New Brunswick, N. J.

[2] Reference is made by number to "Literature cited," p. 139–140.

[3] Frosch, P. Zur Frage der Reinzüchtung der Amöben. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 21, No. 24/25, p. 926–932. 1897.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 3
Oct. 18, 1915
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This was prepared by adding 100 gm. of Penn clay loam soil to 1 liter of a 10 per cent hay infusion plus 0.5 per cent of egg albumin, which the writers had previously found to be best adapted to the large and rapid development of protozoa in such soil (6).

The method of procedure was as follows: The numbers of protozoa in the stock culture solution were first counted by the new method described in a previous paper (6) and recorded under classes of (1) flagellates, (2) small ciliates (12 to 20μ), and (3) large ciliates (25 to 60μ). No amœbæ developed in the short period of incubation. Ten c. c. of the culture solution were then placed (by means of a sterile pipette) on filter paper, previously sterilized with alcohol, and allowed to filter through for one minute. The protozoan content of the filtrate was then recorded in triplicate and the filtrate incubated for five days at 22° C., in order to allow the excystment of any encysted forms. The filtration and incubation processes were then repeated, if necessary, until all the living protozoa of the desired type had been separated out. The filter paper was used in from one to five different thicknesses (Schleicher and Schüll's No. 589). The results are recorded in Table I.

TABLE I.—*Number of protozoa per 10 c. c. of filtrate through varying thicknesses of filter paper*

Number of filter papers.	Sample No.	Number of flagellates.	Number of small ciliates, 12–20μ.	Number of large ciliates, 25–60μ.	Total.
0[a]	1	106, 250	53, 125	42, 500	201, 875
	2	127, 500	42, 500	31, 875	201, 875
	3	85, 000	21, 250	81, 875	188, 125
Average		106, 250	38, 958	52, 083	197, 292
1	1	63, 750	53, 125	0	116, 875
	2	63, 750	31, 875	0	95, 625
	3	74, 375	31, 875	0	106, 250
Average		67, 293	38, 958	0	106, 246
2	1	53, 125	31, 875	0	85, 000
	2	53, 125	21, 250	0	74, 375
	3	73, 750	21, 250	0	95, 250
Average		60, 416	24, 742	0	85, 208
3	1	53, 125	10, 625	0	63, 750
	2	53, 125	10, 625	0	63, 750
	3	63, 750	10, 625	0	73, 750
Average		56, 666	10, 625	0	67, 083
4	1	10, 625	0	0	10, 625
	2	10, 625	0	0	10, 625
	3	10, 625	0	0	10, 625
Average		10, 625	0	0	10, 625
5	1	None.	None.	None.	None.
	2				
	3				
Average					

[a] Stock protozoan solution.

It will be observed from Table I that the large ciliates are not able to pass through the filter paper at all, which fact is in agreement with the experience of Russell and Hutchinson (9, 10). The noteworthy feature, however, is that the number of small ciliates decreases rapidly in increasing the thicknesses of the filter paper from two to four. Thus, with four thicknesses of filter paper all of the ciliates found in the solution employed were separated from the flagellates. Likewise it was a simple matter to separate the small from the large ciliates. In this way it becomes possible to employ mass cultures of flagellates, small ciliates, or large ciliates, as may be necessary in the problems indicated at the outset.

In an effort to determine the effect of filtration on the separation of soil protozoa from bacteria, a bacterial count was made of the stock-culture solution previously employed, known to contain soil microorganisms. Ten c. c. of this solution were then filtered through five thicknesses of sterilized (with alcohol) filter paper (S. & S. No. 589). The residue on the filter paper, consisting of all of the protozoa originally present, together with some adhering bacteria, was then plated out on Lipman and Brown's (7, p. 132) synthetic agar. The bacterial count showed that 90 per cent of the bacteria had passed through the filter paper (after making due deduction for contamination from the air by exposing agar plates for the same length of time as was necessary for filtration), thus leaving the protozoan residue comparatively free from bacteria.

This method in all probability would not allow complete separation of the protozoa from the bacteria. Consequently the work was not carried out any farther. However, this method, because of its rapidity and simplicity, might prove of value in investigations concerned with the effect of protozoa on mixed but not on pure cultures of bacteria.

While these preliminary experiments do not warrant any definite conclusions, they are, nevertheless, indicative of some of the difficulties which the soil protozoologist encounters.

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JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., OCTOBER 25, 1915 No. 4

EFFECT OF TEMPERATURE ON MOVEMENT OF WATER VAPOR AND CAPILLARY MOISTURE IN SOILS

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INTRODUCTION

An investigation of the influence of temperature on the various physical processes in the soil was undertaken by the writer at the Michigan Agricultural Experiment Station. One of the phases of this investigation, the effect of temperature on the movement of water vapor and capillary moisture in soils, will be considered in the present paper.

MOVEMENT OF MOISTURE FROM WARM TO COLD COLUMN OF SOIL OF UNIFORM MOISTURE CONTENT

A rise of temperature decreases both the surface tension and the viscosity of water to the extent shown by the data in Table I.

TABLE I.—*Relation of temperature to the surface tension and viscosity of water*

Temperature.	Surface tension.	Viscosity.
°C.		
0	100. 00	100. 00
10	97. 96	73. 32
20	94. 32	56. 70
30	91. 62	45. 12
40	88. 46	36. 96
50	85. 52	30. 17

It will be noted that the degree of diminution with rise in temperature is considerably greater in the case of viscosity than in that of surface tension.

During the warm part of the year the soil at the upper depths maintains a rather marked temperature gradient which reverses itself between day and night to the depth that the diurnal amplitude of temperature oscillation extends. This diurnal change of temperature gradient occasions an alteration in surface tension and viscosity of the soil moisture, the amount depending upon its variation at the different depths. Since capillary action is said to depend upon surface tension and facility of movement upon viscosity, there should occur an up-

ward and downward movement of moisture as the temperature gradient changes diurnally. During the day, for example, the temperature of the soil is highest at the surface and diminishes with depth; the surface tension and the viscosity of soil moisture are lowest at the surface and rise with depth; consequently, the movement of moisture should be downward. During the night the reverse is true; the soil temperature is lowest at the surface and increases with depth; the surface tension and the viscosity of the soil water are greatest at the top and diminish downward with increase of temperature; hence, the water translocation should be upward.

These considerations are *a priori* deductions from the laws of surface tension and viscosity in their relation to temperature. Whether or not

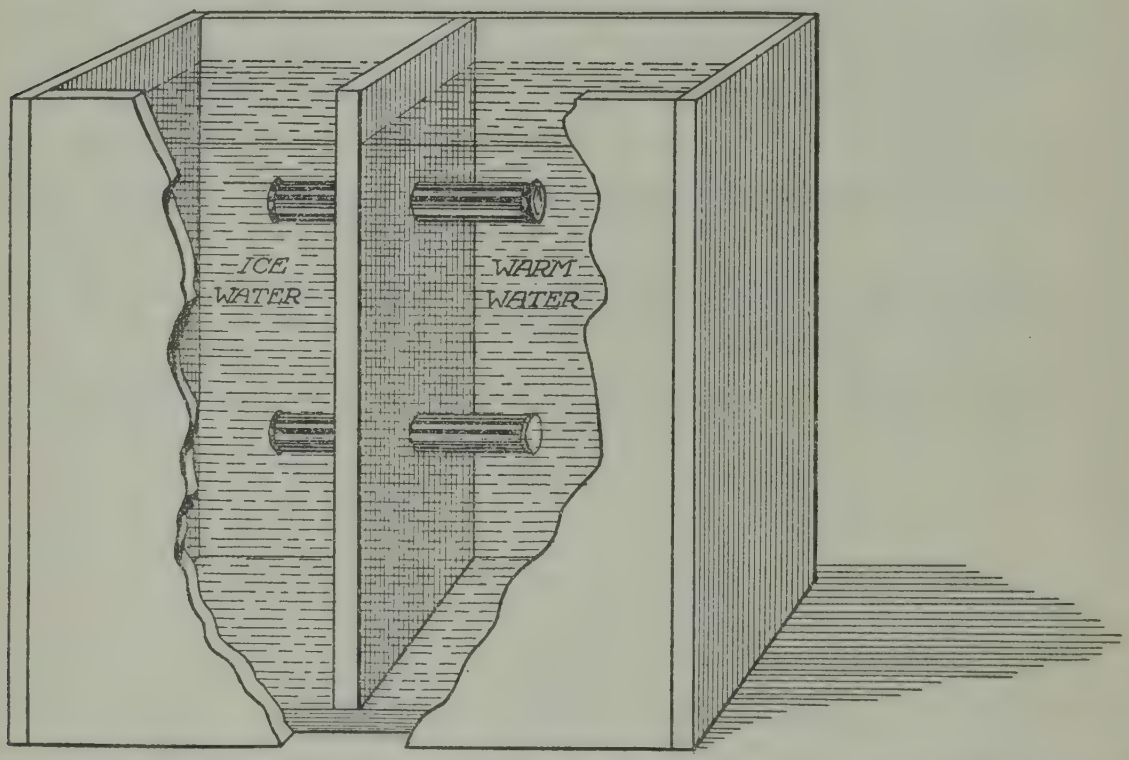

FIG. 1.—Apparatus for determining thermal translocation of soil moisture when the column of soil lay horizontally.

they are valid, however, has heretofore not been known, since there appear to be no experimental data bearing directly upon the subject.

With the object of obtaining this important and much desired information, an investigation of the problem was undertaken. The general method of procedure consisted of placing soils of different but uniform moisture content in brass tubes 8 inches long and 1½ inches in diameter, closing both ends with solid rubber stoppers, and keeping one half of the soil column at a high temperature and the other half at a low temperature for a certain length of time, then determining the percentage of moisture of the two columns and attributing any difference in water content to thermal translocation. There were only two amplitudes of temperature employed, 0° to 20° and 0° to 40° C.—i. e., one half of the soil column was kept at 0° and the other half at 20° and

40° C. For producing these temperature amplitudes wooden boxes were used which contained melting ice and warm water in separate boxes or compartments the temperatures of which were maintained constant by the addition of ice and hot water, respectively.

The movement of moisture from warm to cold soil was studied in two different ways: (1) When the column of soil lay horizontally and (2) when it stood vertically. For the first case, the wooden boxes used were 22 inches long, 10 inches wide, and 20 inches deep, having wooden partitions in the center which contained perforations of the size to fit the tubes (fig. 1). One compartment contained melting ice and the other water at the required temperature. To prevent any exchange of water between the two compartments, the edges of the partition and the

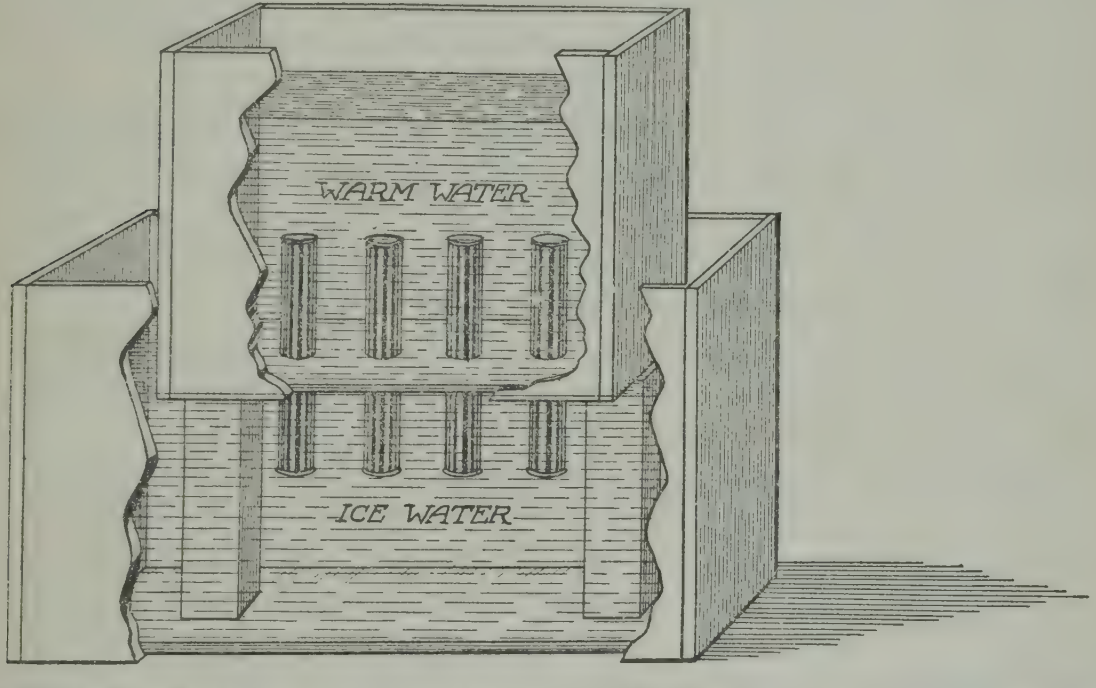

FIG. 2.—Apparatus for determining thermal translocation of soil moisture when the column of soil stood vertically.

holes through which the tubes passed were made water-tight by means of paraffin.

For the second study, the employment of two boxes was necessary (fig. 2). One box, which contained melting ice, was 24 inches long, 10 inches wide, and 13 inches deep. The other box, which contained water at the desired temperature, was 13 inches long, 7 inches wide, and 11 inches deep, and was placed inside the first box. The bottom of the small box was supplied with holes the exact size of the tubes, which were then placed in the holes and the crevices surrounding them sealed with melted paraffin to make the small box waterproof. The inner box was then put upon supports in the large box and was filled with water kept at the desired temperature. The outer box was filled with ice up to and touching the bottom of the inner box. All the boxes were well insulated, and since they were big and contained large volumes of water, the temperature could be kept to within small variations for long

periods. The water was stirred occasionally to maintain uniformity of temperature throughout its mass.

The temperature amplitudes employed are within the upper limit of the diurnal amplitudes of temperature at the upper depths in the soil, but they are too high for the range of temperature that exists at any one time between the various adjacent depths.

The duration of each experiment was about eight hours. This time limit was calculated to represent approximately the length of period that the day and night soil temperature gradient is most marked.

The effect of temperature on the movement of moisture in soils of uniform moisture content was investigated in five diverse classes of soil: Miami light sandy loam, Miami heavy sandy loam, Miami silt loam, Clyde silt loam, and Miami clay. Each soil contained a large number of different moisture contents. These various moisture contents in each soil ranged from very low to very high.

To procure a very uniform moisture content throughout the soil column, each soil, after it was moistened to the desired degree, was passed through a sieve and then mixed thoroughly. It was then placed in the tubes and packed uniformly by allowing the tubes to fall in a vertical position from a certain height a definite number of times.

At the end of each experiment the warm column was separated from the cold column of soil by means of a spatula. This was done by drawing out all the soil from that warm section of the tube which extended up to the plane of the partition and allowing for the cold column all the soil that was contained in that cold section of the tube up to the other plane of the partition, and also that portion of the soil contained in the tube under the hole of the partition. This last part of the soil was accorded to the cold column of soil because its temperature is intermediate between the opposite temperature extremes, and it was desired to make the lines of demarcation between the two columns of soil as prominent and distinct as possible. The moist soils were dried in an electrical oven for about 20 hours at a temperature of 105° C., and the percentage of moisture content was calculated on the dry basis. The weights were always determined on a sensitive chemical balance.

The fact has been mentioned that the movement of moisture from a warm to a cold column of soil was studied in two different ways: (1) When the column of soil lay horizontally and (2) when it stood vertically. The data obtained from both series of experiments show that if the same percentages of moisture were employed practically the same results would be obtained, no matter whether the soil columns remained in the horizontal or vertical position. For the sake of brevity and simplicity of presentation, therefore, only the results of the series of experiments wherein the soil column was held in the vertical position will be presented here. These experimental data, together with their diagrammatic representations, are submitted below. Table II gives the

various moisture contents of the different soils and the percentage of moisture moved from the column of soil at 20° to the column of soil at 0° and from the column of soil at 40° to the column of soil at 0°. The percentage of moisture moved represents the difference between the percentages of moisture found in the cold and the warm columns of soils, respectively, at the end of the experiment; at the beginning of the experiment the moisture content was the same in both columns of soil. Figure 3 represents these data in a graphical form.

TABLE II.—*Movement of moisture from a warm to a cold column of soil of uniform moisture content*

Kind of soil.	Percentage of moisture in soils.									
Sandy loam:										
Beginning of experiment	2.29	3.86	6.45	7.50	8.48	9.95	10.94	13.75	15.96	.....
Movement from 20° to 0° C.	.102	.296	.792	.900	.530	.520	.466	.340	.100	.....
Movement from 40° to 0° C.	.410	1.064	1.97	2.882	1.715	1.467	1.30	.97	.30	.....
Heavy sandy loam:										
Beginning of experiment	4.20	6.52	9.08	10.42	12.43	14.02	16.03	.....	.....	.....
Movement from 20° to 0° C.	.160	.631	.930	.721	.582	.491	.21	.....	.....	.....
Movement from 40° to 0° C.	.59	1.75	3.02	2.40	1.98	1.40	.42	.....	.....	.....
Silt loam:										
Beginning of experiment	4.29	8.06	9.76	11.28	14.441	15.95	17.63	19.30	21.42	23.51
Movement from 20° to 0° C.	.138	.736	1.024	1.180	1.190	1.10	.85	.48	.35	.21
Movement from 40° to 0° C.	.471	1.98	2.65	3.276	3.68	3.58	2.60	1.75	1.02	.45
Clyde silt loam:										
Beginning of experiment	7.56	12.51	14.98	17.59	18.80	21.55	22.76	29.98	34.57	.....
Movement from 20° to 0° C.	.122	.46	.89	.96	1.07	.99	.83	.62	.20	.....
Movement from 40° to 0° C.	.409	1.72	2.07	2.45	3.27	2.82	2.30	1.36	.51	.....
Clay:										
Beginning of experiment	9.70	18.38	19.29	20.69	22.98	29.88	.....	.....	.....	.....
Movement from 20° to 0° C.	.248	.72	.99	.73	.70	.681	.....	.....	.....	.....
Movement from 40° to 0° C.	.672	2.60	3.29	2.50	2.12	1.88	.....	.....	.....	.....

The foregoing data present many important and remarkable facts. First of all, they show most emphatically that the *a priori* prediction regarding the thermal movement of moisture as deduced from the laws of surface tension and viscosity in their relation to temperature is not strictly realized. According to these laws, the amount of water moved from a warm to a cold column of soil should be the same for all moisture contents, provided the soil mass exerts no influence upon water; inasmuch, however, as the soil does exert an adhesive force upon water, the thermal translocation of moisture should increase with a rise in water content. Instead, the percentage of water moved from a warm to a cold column of soil at both temperature amplitudes increases regularly and rapidly with an increase in moisture content in all the different types of soil until a certain moisture content is reached, and then it begins to decrease with a further rise in the percentage of water. The results then plot into a parabola, with a maximum point instead of a straight line. This maximum point of water thermal translocation is significant in at least two ways: (1) It is quantitatively about the same for all classes of soil and qualitatively the same for both amplitudes of temperature; and (2) it is attained at entirely different moisture contents in the various soils and at a comparatively low percentage

of moisture. On referring to the data in Table II it will be seen that the maximum thermal water transference at the amplitude of 20° C. is 0.90 per cent for light sandy loam, 0.93 for heavy sandy loam, 1.19 for silt loam, 1.07 for Clyde silt loam, and 0.99 for clay; at the temperature amplitude of 40° it is 2.88 per cent for light sandy loam, 3.02 for heavy sandy loam, 3.68 for silt loam, 3.27 for Clyde silt loam, and 3.29 for clay. It should be noted that the percentage of thermal

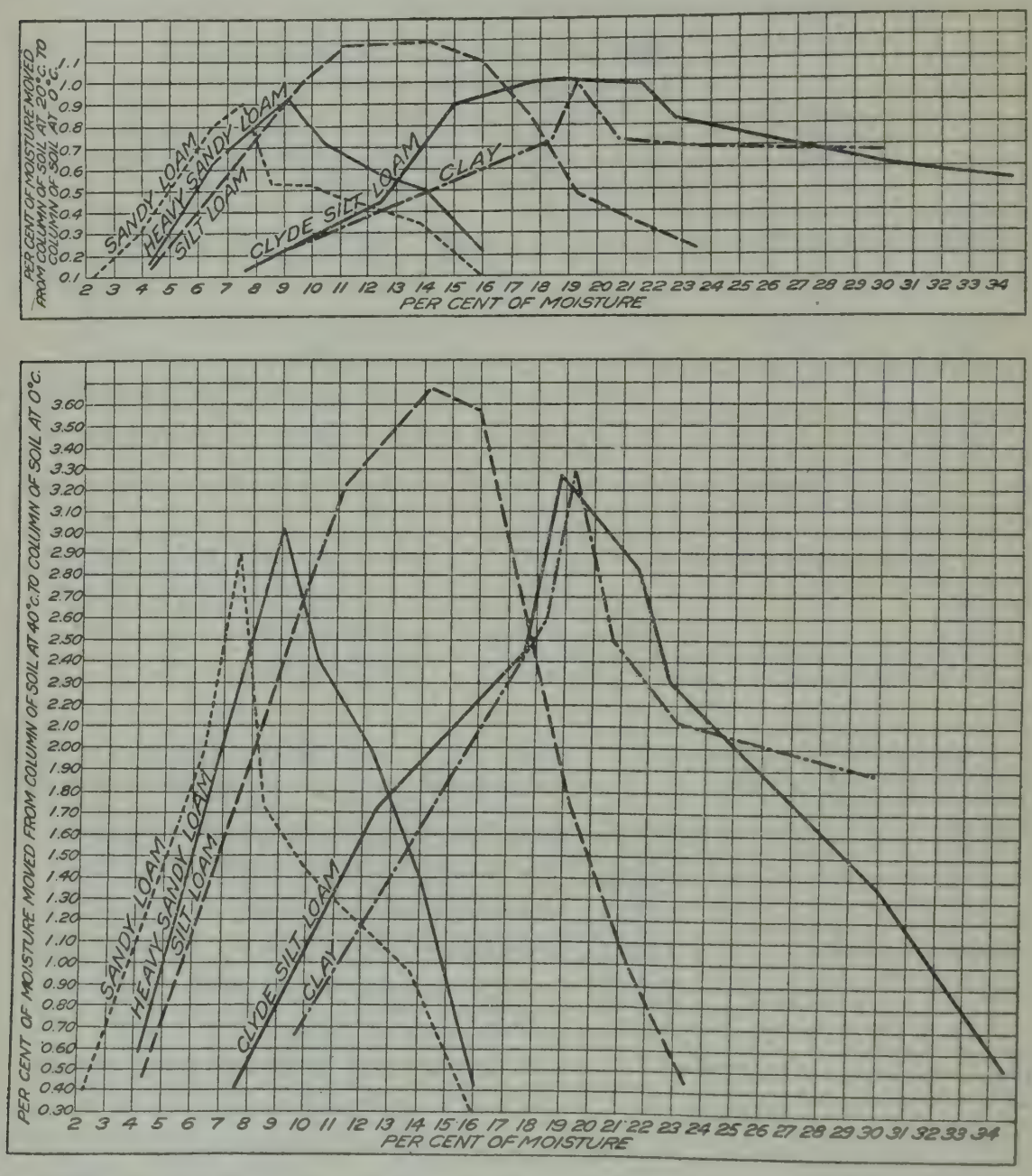
PER CENT OF MOISTURE MOVED FROM COLUMN OF SOIL AT 40°C. TO COLUMN OF SOIL AT 0°C.
PER CENT OF MOISTURE
SANDY LOAM
HEAVY SANDY LOAM
SILT LOAM
CLYDE SILT LOAM
CLAY

FIG. 3.—Curve showing the movement of moisture from a warm to a cold column of soil of uniform moisture content.

motion of water increases more than proportionally with temperature. The temperature of 40°, for instance, is only twice as great as 20°, while the percentage of moisture moved is three times greater in the former case than in the latter. The water content of the various soils at which the maximum thermal translocation occurs is 7.50 per cent for light sandy loam, 9.08 for heavy sandy loam, 14.21 for silt loam, 18.80 for Clyde silt loam, and 19.29 for clay.

Obviously, then, the maximum thermal water movement depends upon a definite condition of moisture of any particular soil; a deviation from this definite degree of moisture in either direction causes a decrease in thermal movement of water. Since this definite percentage of moisture at which the greatest quantity of water is able to move from a warm to a cold column of soil appears to be a specific constant or characteristic of the various soils, it is proposed to designate it as "thermal critical moisture content." A thermal critical moisture content may be defined, then, as that percentage of moisture in soil which allows the greatest amount of water to move from warm to cold soil at any amplitude of temperature.

A further examination of the preceding experimental data shows that the thermal movement of moisture is extremely sensitive to the amount of water present in a soil. It will be noted that by increasing or decreasing the percentage of soil water by small degrees, the thermal movement varies very markedly in either direction. From this it follows that the thermal critical moisture content must be quite definite, and in order to obtain it absolutely, the percentage of soil moisture near the point of maximum thermal movement must be increased by small amounts. This applies especially to the light sandy soils, in which the sensitiveness appears to be more marked and the range more limited. If the increase in percentage of moisture content took place in this soil by 0.1 instead of 1.0 per cent, the maximum thermal translocation would probably have been as high as that of the other soils. It is possible, however, that the value obtained is about the upper limit for this soil and consequently for all soils of its type.

The diminution of the thermal translocation of water with a decrease in moisture content from the point of thermal critical moisture content might be anticipated, but the decrease of water movement with further increase of moisture content after the point of thermal critical moisture content was not expected. Indeed, it was at first thought that the movement would be greater at the highest moisture content because there would also occur a gravitational movement. When soils contain as high as 35 and 30 per cent of moisture, as did the Clyde silt loam and the clay, respectively, and when one half of their column is kept at 40° and the other at 0° C. for eight hours, such expectation as the above is not at all unnatural. Instead, the water movement at these highest moisture contents is very low and in descending order and the cessation of diminution is not as yet reached. These results go to show, then, in a most striking manner that the soils possess a very great attraction for water and that their requirements for water to satisfy their attractive forces before free movement of water can take place are, indeed, high. Until the point is reached where gravitational movement occurs, the moisture in the soil is held by a force of great magnitude.

Now, the next question is, How may this peculiar thermal translocation of water be explained? What are the causal agents which bring it about?

As already stated, it is not entirely due to the surface tension and viscosity of the soil water, for if that were the case then the movement should have followed a different course. If the soil exerted no adhesive force for water, the amount of moisture moved from a warm to a cold column of soil should be the same for all moisture contents, provided the force of gravity is eliminated for any particular amplitude of temperature. But since the soil does exert a strong adhesive force for water, the thermal motion of water should follow a straight line with rise in moisture content for any given difference in temperature. Instead, the results plot into a parabola. Evidently there must be another explanation for the phenomena.

The best explanation suggested appears to be founded upon the following four assumptions: (1) The soil possesses an attractive power for water and holds it with a great adhesive force; (2) these attractive and adhesive forces decrease with increase in temperature; (3) the surface tension or cohesive power of the liquid also diminishes with rise in temperature; and (4) the force due to the curvature of the water films between the soil grains, which are known as capillary films, decreases with increase of water content.

All these four assumptions appear to be correct. The validity of the third and fourth is generally recognized and consequently needs no further discussion. The validity of the first is also universally accepted: That the soil possesses an attractive power for water can hardly be denied; that the soil holds the water with a great adhesive force is evidenced by the great difficulty experienced in attempting to separate the one from the other. Indeed, this adhesive force is so great that no method as yet has been devised either to execute a complete separation of the two components or to measure with any degree of precision its magnitude. The researches of Lagergren (8),[1] Young,[2] and Lord Rayleigh (10) indicate, however, that this force may be of an order of magnitude from 6,000 to 25,000 atmospheres.

The great attractive and adhesive forces which the soil exerts upon water are further illustrated by the researches of Briggs and McLane(3) on the moisture equivalent and by those of Briggs and Shantz (4) on the wilting coefficient of plants. By whirling wetted soils in a rapidly revolving centrifuge fitted with a filtering device in the periphery and developing a force equivalent on the average to 3,000 times the attraction of gravity, Briggs and McLane found that some clay soils would still contain about 50 per cent of water. The studies of Briggs and McLane on the wilting coefficient of plants show that plants would wilt and die in clay soils even

[1] Reference is made by number to "Literature cited," p. 172.

[2] Cited by Minchin, G. M. Hydrostatics and Elementary Hydrokinetics, p. 311, London, 1892.

when the moisture content was still about 30 per cent. As the water content increases, these attractive and adhesive forces decrease.

Of all the four assumptions the correctness of the third—namely, that the attractive and adhesive forces decrease with temperature—may be doubted by many and challenged by a few. The theoretical and experimental evidences, however, are overwhelmingly in its favor. According to the law of kinetic energy, the attractive and adhesive forces of solids for liquids and gases or vapors should decrease with rise in temperature. The investigations upon the absorption of gases and vapors at different temperatures show this to be the case. The work of De Saussure (11) and Von Dobeneck (6) upon the absorption of gas by different solid materials, and the researches of Knop[1] and Ammon (1) upon the absorption of water vapor by soil, seem to show conclusively that the absorptive power of diverse solid materials for gases and water vapor decreases with increase in temperature. The only evidence which is contrary to the above is that obtained by Hilgard (7, p. 198) on the absorption of water by dry soils from a saturated atmosphere. Hilgard's results show that the absorption of water vapor by soils increases with rise in temperature. The results obtained by the several investigators mentioned, as well as new evidence which will subsequently be presented, tend to throw considerable doubt on the correctness of Hilgard's data. Hence, it can safely be asserted that the third assumption is correct.

Bearing these postulates in mind, the phenomena of thermal water translocation observed may be explained as follows: The soil with the lowest moisture content holds the water with a force of great magnitude. When the temperature of a column of this soil is uniform throughout, the adhesive and attractive forces are at an equilibrium. When one half of this column of soil is heated to 40° and the other half to 0° C., this equilibrium is disturbed. The attractive and adhesive forces of the soil for water and the cohesive power or surface tension of the soil water are decreased in that portion of the soil column which is maintained at 40° and increased to a corresponding magnitude in that portion of the soil column which is kept at 0° C. The cold column therefore exerts a pull and draws water from the warm column in amount depending upon the quantity that the latter is willing to give up. Since the soil possesses a great attraction for water, which attraction varies with the diverse classes of soil, and inasmuch as this attractive force is not satisfied at the low moisture content, the warm soil parts only with a small amount of its water. Hence, the amount of water moved from the warm column to the cold column of soil is small.

At the next higher moisture content the attractive power of the soil for water is further satisfied and the total water content is held with less force. When a column of this soil is kept at the same amplitudes of temperature as above, the decrease and increase of the adhesive

[1] Cited by Johnson, S. W. How Crops Feed. p. 164. New York [1870].

and cohesive forces, due entirely to temperature, between the warm and cold columns of soil are equal in amount, as in the soil with the lowest moisture content. Water, therefore, tends to move from the warm to the cold soil. Inasmuch as the attraction of the soil has been further satisfied and the water films further thickened, the pull of the cold soil, due only to the attractive forces of the soil for water, is decreased; on the other hand, the ease with which the warm soil gives up moisture is increased. The result is that even though the total effective pull (composed of the increased surface tension of water, the increased attractive adhesive forces of soil for water, and the force of the curvature of the capillary films) of the cold soil with the high moisture content is less than that of the soil with low moisture content, the greater ease with which the warm soil with high water content parts with moisture enables the reduced effective pull to draw more water from the warm to the cold side. As the moisture content of the soil is continually increased, its attractive power is satisfied and the curvature of the capillary films decreased correspondingly. The total effective pull of the cold column of soil is continually decreased, but the ease with which the warm column of soil gives up moisture is also continually increased, so that the thermal translocation of water is constantly increased with rise in moisture content.

Finally, a degree of moisture content is reached in which the effective pull of the cold column of soil is able to extract the greatest amount of water from the warm column of soil. This degree of water content is the thermal critical moisture content. At this point the attractive power of the soil for water is considerably satisfied but is far from being entirely appeased; the total effective pull of the cold column of soil is also considerably less than that of the preceding columns of soil, but the warm column yields water to this pull with such ease that there occurs a maximum thermal water translocation. Inasmuch as the water-attractive power is different for the various kinds of soils, this thermal critical moisture content is of necessity also different. After this thermal critical moisture content is reached, the effective pull of the cold column of soil is further decreased with a continued increase of moisture content. And although the willingness of the warm column of soil to part more readily with moisture is also increased, yet the pull of the cold column of soil is not sufficiently strong to draw it; consequently the thermal movement of water commences to decrease and continues to diminish very regularly and gradually with a continued increase in moisture content. When the highest percentage of water is reached, the warm soil is very willing to part with a very large amount of water, but since the effective pull of the solid soil is reduced almost to a minimum, only a small amount of moisture is drawn from the former to the latter.

The degree of moisture of the different soils could not be further increased, on account of the difficulty of sifting them, and consequently it

can not be stated with certainty whether the thermal movement of water would become zero at a still higher moisture content. From the theoretical point of view, however, it should not become zero, because the pull due to the surface tension of water alone is not affected by increase of moisture content, but remains constant. The portion of pulling force which is decreased constantly with a rise in moisture content is that pertaining to the attractive power of soil for water and to the curvature of the capillary film. At or near the point of saturation the pulling power due to these two factors is probably zero; at this point the soil may be considered to be passive. Any thermal movement of water that takes place at or near the point of saturation is to be attributed to the surface tension of the soil water. If this assumption is correct and if the percentage of moisture moved at the highest moisture contents employed is to be considered as a measure of the amount of thermal translocation due to surface tension of water alone, it will be found that the quantity due to this force is very small indeed. As will be seen from the experimental data, the percentage of moisture moved at both amplitudes of temperature is reduced to an insignificant value at the highest moisture contents.

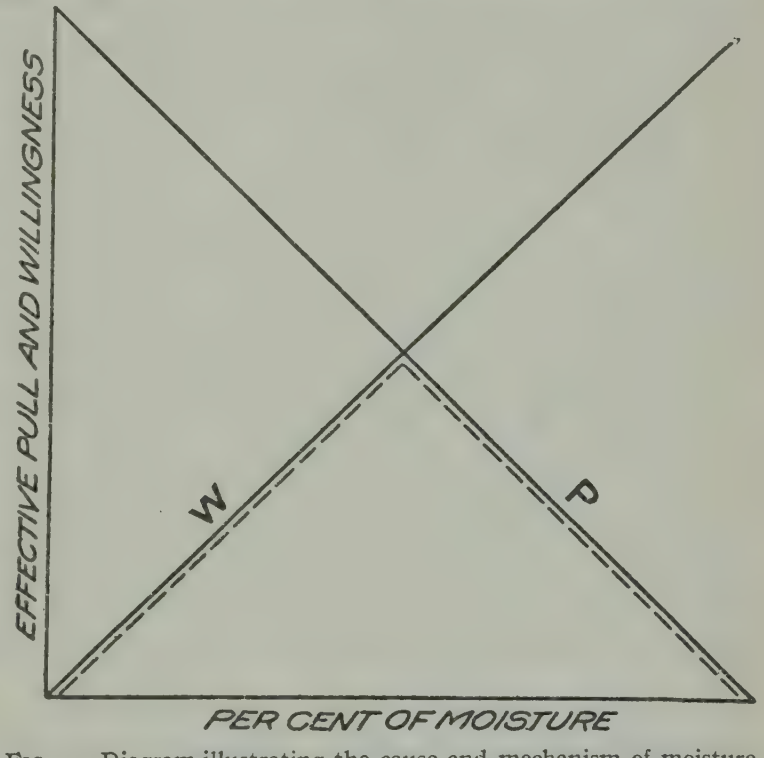

FIG. 4.—Diagram illustrating the cause and mechanism of moisture movement from a warm to a cold column of soil of uniform moisture content.

The foregoing exposition as to the cause and mechanism of the phenomena of thermal water translocation will probably be made clearer by figure 4. This diagrammatic representation, however, by no means pictures the real cause and mechanism absolutely and accurately, but it will serve, it is believed, to make clearer what has already been said.

Let the abscissa represent the effective pull of the cold column of soil and the willingness of the warm column of soil to part with water at a different moisture content, and let the ordinates represent the different percentages of water contained by the soil. By plotting the effective pull and willingness against the moisture content it will be seen that the effective pull decreases and the willingness increases with a rise in moisture content. At the point where the two lines cross probably occurs the

maximum thermal translocation of water. After this point of intersection the willingness of the warm soil to give up water is large, but since the effective pull is being reduced to a minimum the water is not moved. If a parabola is now drawn along the lines WP, with its maximum value at the point of intersection, then this theoretical curve will agree almost perfectly with the real one in figure 3.

The serious fault with the above illustration (fig. 4) is that the total effective pull tends to become zero, and theoretically this should not be the case, because while the pull due to the attractive power of the soil for water and to the curvature of the capillary films will ultimately become zero, the pull due to the increased surface tension of the soil water should not become zero, but should remain the same for all moisture contents. Hence, figure 4 illustrates more correctly only the thermal translocation of the water as due to all the other forces except the surface tension of water.

The next important question to consider is the mode and amount of thermal translocation of water in field soils as suggested by the foregoing laboratory experimental data. Under field conditions the soil moisture exists practically always in a gradient form. As the water content tends to decrease upward from the water level, the forces due to the curvature of the capillary film and to the attractive power of the soil for water increase correspondingly; consequently the pull is upward. The soil temperature also exists in a gradient form, but this reverses itself diurnally and therefore modifies these pulling forces. During the day the temperature at the upper depths is higher than that below; the attractive and adhesive forces of the soil for water and the surface tension of water are decreased, so that the total upward effective pull is diminished correspondingly. Inasmuch as the temperature below is less than that above, the effective pull due only to the increased attractive and adhesive forces of the soil for water and to the surface tension of the soil water should occasion a downward movement of moisture. Since, however, the water-attractive forces of the soil below are more satisfied than those of the soil above, the downward pull due only to the attractive adhesion and surface tension as increased by a lower temperature is very small in comparison with the upward pull. Hence, during the day the moisture movement is upward. During the night nearly all of the above forces act in a parallel direction and favor an upward movement. Therefore, the thermal movement of moisture in soils is always upward and never downward.

The extent to which moisture will move during the night from the warmer soil below to the colder soil above will depend (1) upon the soil temperature gradient—that is, upon the difference in temperature of the various adjacent depths—and (2) upon the gradient or amount of moisture content at the various depths. In the preceding series of

experiments the temperature amplitudes of 20° and 40° C. were employed. In nature, however, so large and sharp variations in temperature between adjacent depths never occur during the night; they do occur, however, at the upper depths between day and night. Soil-temperature investigations which are being conducted at this Station show that in the early morning, when the temperature gradient is most marked, the temperature of the bare mineral soils increases sometimes in the summer and fall at the average rate of about 2° or 3° for each inch of depth down to about 4 inches, and then this rate becomes less. In cropped soils, where the temperature remains more constant, this rate of increase of temperature with depth is still less. Hence, the amount of thermal translocation of water that would occur during a single night would be very small. On the other hand, the maximum thermal translocation of water obtained in the preceding series of experiments was procured from a column of soil with uniform moisture content. As will be shown subsequently, there is no doubt whatever that this maximum thermal translocation of water in the various soils would have been far greater if the moisture content of the cold column was less than that of the warm column of soil. In nature, as already mentioned, the moisture exists in a gradient form; consequently the movement of water is upward and the forces of the factors which cause this upward movement are increased during the night. Therefore, while the amount of thermal translocation of water during a single night in soils under field conditions may not be as great as that obtained in the foregoing series of experiments, yet it will be quite appreciable; and since the process is repeated, the sum of the translocation for all the nights during the vegetative season will probably be considerable.

The moisture content at which the maximum thermal translocation of water occurs, or what has been designated as the thermal critical moisture content, is very significant and needs further consideration. It would be of very great interest to know, for instance, the thickness of the water film around the particles at this degree of moisture. This thickness could be calculated if all the soil grains were solid and spherical. The particles of the soils used, however—and these are the commoner types of agricultural soils—are neither spherical nor solid. Nearly all the particles in agricultural soils can be said to be irregular in shape. Some of them are solid and enveloped with a colloidal coating; others are compound aggregates, or "crumbs," and are porous; and still others, mainly of the peat nature, are of a sponge structure and are necessarily porous. The particles of a soil or soils may be classified under two categories: (1) Particles which are solid and have only an external surface and (2) particles which are partly or wholly porous and possess both an external and internal surface. In the solid and cleaned surface particles the water film is spread over the surface, but the film of water

envelops theoretically the whole external surface of the solid particles coated with colloids, or the mineral floccules and the organic particles; and water also permeates their internal surface. The single solid mineral grains, which may compose the compound particles, may be cemented together in a way analogous to that found in a piece of sandstone, in which case the water exists only in the interstices and not as a complete film around each particle. Furthermore, whether the soil grains are solid or spherical or compound and porous the water film is not uniform in thickness over the entire inner surface of the soil mass, but thickens more at the capillary angles between the particles.

In view of these considerations, therefore, it was considered useless to attempt to compute the thickness of the film, as many investigators have done. Furthermore, in view of the nature of the soil particles, as discussed above, it does not appear strictly proper to define the capillary water in the soil as a thin film overspreading the particles and thickened into a waistlike form at their points of contact. Hence, a new definition of capillary water is needed.

If we are to accept the theory which has been used to explain the foregoing phenomena of thermal translocation of water, that the soil possesses a very great attraction for water, that this attractive force is different for various soils, that it decreases with a rise in moisture content, and that it is completely satisfied at a considerably high moisture content, then our present views concerning the movement of capillary water in moist soils need modification. The present theory regarding the capillary movement of water consists of an analogy from the rise of water in capillary tubes. The interstitial spaces of a soil mass are considered as forming channels analogous to capillary tubes and are often designated as bundles of capillary spaces. The capillary water is believed to exist as surface films around the particles and as capillary films in the capillary spaces between the particles, and its movement is said to depend entirely upon the curvature of the capillary films. When a dry soil, for instance, is well moistened and brought to equilibrium, the water films are thick and the curvature of the capillary films small, and there will be no further capillary attraction of water if this soil is brought in contact with water. If this soil is allowed to dry at the top, the surface films become thinner and the force of the capillary films increases in direct ratio with their degree of curvature; hence, there will be a pull of water from the thicker surface films and less curved capillary films below toward the surface.

It is obvious that with this theory of capillary movement of water the whole cause of the capillary movement of water in a moist soil is attributed to the curvature of the capillary films between the particles, and the moist soil is considered as being passive, inactive, and exerting no influence whatever upon the movement of water. Indeed, Briggs

and Lapham (2), in trying to explain the differences in capillary action in dry and moist soils, make the following statement:

> In a moist soil, however, we have quite another condition. A film of the liquid covers all the surfaces of the soil grains. Since this film, once established, is maintained in a saturated atmosphere, it follows that the solid air and solid liquid surface forces no longer play any part in the capillary movement, which is produced entirely by the air liquid surface force and is opposed only by the weight of the liquid column.

In view of this general belief, Briggs, as well as other investigators, has tried to alter the properties of the soil water by increasing its surface tension, etc., with the object in view of increasing its capillary action.

If it were true that as long as a thin film of water is maintained in a damp or slightly moist soil the soil material itself exerts no longer any influence upon the movement of capillary water, then the preceding theory might be true. But we have seen in postulate 1 (p. 148) that the soils, and especially those rich in colloidal material, possess a very great attractive power for water, that this attractive power is satisfied only at a considerably high moisture content, that as long as it is not satisfied the soils will continue to take up water, and that they hold the water with a force of great magnitude. In view of the considerations presented in this postulate, and in view of the fact that the preceding thermal movement of water appears to be controlled by the attractive forces of the soil for water, it seems wrong to consider the soil material in moist condition as a static, passive, inactive, and irresponsive skeleton upon which the liquid plays its rôle. The solid material in moist condition short of saturation is dynamic and not static in respect to moisture movement. Hence, the capillary movement of water should not be attributed entirely to the forces exerted by the curvature of the capillary films, but also to the forces exerted by the unsatisfied attractive power of the soil for water. When a moist soil, therefore, begins to lose water at the surface, two effects are produced: (1) The attractive forces of the soil for water are increased and (2) the curvature of the capillary films is increased. Both of these effects exert a pull on the moist soil below and tend to draw water to the surface. As to which one of these two forces exerts the greatest pull it is impossible to say, because there is no way of measuring them. It is certain, however, that the force resulting from the attractive power of the soil for water must be very considerable, and probably it is the predominant of the two.

It might be argued that the preceding phenomena of thermal translocation of water could be explained entirely by the film theory without having to resort to the conception of the attractive forces of the soil. Such contention, however, can not be maintained, first, because it can not be conceived that the tension of the capillary films is operative and effective at such high moisture contents employed and, second, because the fact remains, nevertheless, that the soil exerts a pull owing to its

attractive forces for water, as has been abundantly proved. Furthermore, if it is maintained that the attractive forces of the soil for water are satisfied as soon as the soil is merely damped, then why should the soil hold additional large amounts of water with such a great force that it is impossible to extract it with mechanical means? It seems reasonable, therefore, to believe that if the soil holds large amounts of water with a great force, it should attract or absorb it with a force of equal magnitude.

MOVEMENT OF MOISTURE FROM A MOIST AND WARM COLUMN TO A DRY AND COLD COLUMN OF SOIL WITH AN AIR SPACE BETWEEN THE TWO COLUMNS

In the preceding section the thermal translocation of water was considered as occurring as water-film phenomena. There is still another way in which this thermal movement of moisture might take place: By vaporization and condensation of soil water from a point of high temperature to a point of low temperature. It is well known that water undergoes a transformation into the vapor state upon the application of heat, and the quantity of liquid vaporized increases with a rise in temperature. One of the remarkable characteristics of aqueous vapor is its sensitiveness to heat, changing from a gaseous to a liquid state, and vice versa, with very small variations in temperature. An excellent paradigm of this latter fact is the relative humidity of the air at different temperatures.

Since the temperature gradient of the soil reverses itself during the night—that is, it increases with depth—it is believed that there is a rising of vapor or moist air from the warmer soil below to the colder soil above, where the moisture is condensed. As a manifest proof of this theory, the morning dew is cited. It is concluded, therefore, that a large part of the water movement in soils is due to this process.

There appear to exist no direct experimental data as to whether or not there really is a translocation of moisture in soil at night, due to upward movement of the moist warm air and the condensation of its moisture at the cold soil above. Practically all of our present knowledge upon the subject consists of theoretical deductions from practical observations.

With the object of obtaining experimental evidence upon the subject the following investigation was performed. Into brass tubes 8 inches long and 1½ inches in diameter was placed moist soil at one end and dry soil at the other and the two columns separated by an air space. This air space was one-fourth of an inch in height and 1½ inches in diameter and was produced by placing between the two columns of soil a ring of cork, the two sides of which were closed with wire gauze that acted as supports of the two soils and prevented their particles from coming in contact. The tubes were then placed horizontally in the boxes shown

in figures 1 and 2. That part of the tubes which contained the moist soil was kept at 20° and 40° and the part which contained the dry soil was maintained at 0° C. The experiment was allowed to run about eight hours. If during this period the dry and cold soil gained any moisture, it obtained it by the condensation of vapor which was produced at the warm and moist soil. Since the dry soil possesses a high absorbtive power for water, it was assumed that it abstracted the vapor from the air space and that this air space was thus prevented from attaining an equilibrium. Five different classes of soil were used: Quartz sand, Miami light sandy loam, Miami silt loam, Clyde silt loam, and Miami clay. The moisture contents employed for each soil were three: Low, medium, and high. The percentage of moisture moved from the warm and moist column of soil to the cold and dry column of soil represents the difference between the percentages of moisture found in the dry soil at the beginning and end of the experiment. The results obtained are presented in Table III.

TABLE III.—*Movement of moisture from a warm and moist column of soil to a cold and dry column of soil, with an air space between the two columns*

Kind and temperature of soil.	Percentage of moisture in soil.		
Quartz sand:			
At beginning of experiment	2.90	6.83	13.52
Movement from moist column at 20° to dry column at 0° C	.051	.046	.048
Movement from moist column at 40° to dry column at 0° C	.286	.280	.294
Sandy loam:			
At beginning of experiment	7.23	10.27	15.82
Movement from moist column at 20° to dry column at 0° C	.0238	.0313	.0246
Movement from moist column at 40° to dry column at 0° C	.211	.253	.223
Silt loam:			
At beginning of experiment	9.16	14.52	16.40
Movement from moist column at 20° to dry column at 0° C	.024	.033	.0273
Movement from moist column at 40° to dry column at 0° C	.278	.273	.288
Clyde silt loam:			
At beginning of experiment	9.85	15.51	22.39
Movement from moist column at 20° to dry column at 0° C	.028	.031	.040
Movement from moist column at 40° to dry column at 0° C	.16	.22	.28
Clay:			
At beginning of experiment	10.77	15.36	20.35
Movement from moist column at 20° to dry column at 0° C	.08	.06	.09
Movement from moist column at 40° to dry column at 0° C	.18	.36	.26

The results in Table III show the most surprising fact that the amount of moisture moved from the moist and warm column of soil to the dry and cold column of soil by vapor is very insignificant. It will be seen that at the temperature amplitude of 40° the quantity of moisture moved is only about 0.25 per cent, and at the amplitude of 20° the value is only about 0.035 per cent. In comparison with the results of Table II, where it is shown that the maximum thermal movement of water at the thermal critical moisture content, when the soil mass is continuous, runs as high as 3.68 per cent in some cases, the above values, due only to vapor movement and condensation, are extremely insignificant.

From these results then it is safe to conclude that the thermal movement of moisture due to distillation is practically negligible, even at such high amplitudes of temperature as 20° and 40° C., which never exist during the night at the different depths on the soil, nor during such a long, continuous period as eight hours. This conclusion is indirectly substantiated by the studies of Buckingham (5) on the loss of soil moisture by direct evaporation from points below the surface. By exposing a surface of water or moist soil to evaporation into a confined space which was in communication with the outside air through a column of soil, Buckingham found that the actual mean rate of loss of water through diffusion of water vapor through soils in still air was very small.

Another noteworthy fact in the foregoing experimental data is that the amount of distillation from moist and warm to the dry and cold column of soil is the same for all moisture contents. This might have been anticipated, since the amount of water vaporized depends principally upon the temperature and is not governed by the amount of water present. On the other hand, if the amount of water present in the soil is extremely small, the water is held by the soil grains with an attraction of great magnitude, causing a lowering of the vapor pressure of the absorbed water film and thereby producing a diminution in the rate of evaporation. Perhaps the water contained in the soil with the lowest moisture content was above the point where this lowering of vapor pressure occurs; and consequently the partial pressure of the vapor in the air space in this soil was the same as in the air space of the soil with the greater moisture contents. Furthermore, the values are so small as to lie within the experimental error, and the method of moisture determination may not be sufficiently sensitive and accurate to show any decreased evaporation by the soils with the lowest moisture content.

In undertaking and performing the foregoing series of experiments it was taken for granted that there really is an upward movement of moist air during the night from the warmer soil below to the colder soil at the surface, where its vapor is condensed. This theory seems to be now very widely accepted, as already stated. The formation of the dew

is attributed by many writers almost entirely to this thermal movement of vapor. Thus, in discussing the subject Hilgard (7, p. 307) states that "dew is formed from vapor rising from the warmer soil into a colder atmosphere, and condensed on the most strongly heat-radiating surfaces near the ground, such as grass; leaves, both green and dry; wood; and other objects first encountering the rising vapor." Farther on he says: "The fact that dew is most commonly derived from the soil could have been foreseen from the other fact, long ascertained and known, that during the night the soil is as a rule warmer than the air above it." Other writers, such as Ramann (9), etc., claim in substance the identical belief.

But really, is there a rising of vapor or warm moist air from the warm soil below to the cold soil above? And is the source of water of the dew ascribable to this soil vapor? During the day the soil receives its heat at the upper surface, and its temperature rises. The heat is conducted downward, and the temperature of the various depths of the soil increases correspondingly. The temperature at the surface continues to increase until a maximum is reached and then begins to decrease. As the temperature increases and moves downward, the soil air expands, and as the volume of the pore space remains constant, it is expelled into the atmosphere. The pressure of the soil air at the different depths tends to be the same at any one time and equal to the atmospheric pressure, provided the communications are ideal. When the temperature at the surface soil is at the maximum, it is generally many degrees higher than that of the air above, amounting sometimes to 30° C. In fact, the air temperature decreases in calm and clear weather with an increase in height at the adiabatic rate of approximately 0.9° per 300 feet. When the temperature of the surface soil and of the air is highest, the atmospheric pressure also tends to be at its minimum, so that the air escapes from the soil with greater facility. After the surface soil attains its maximum temperature and then begins to cool, its air contracts, tends to produce a partial vacuum, and consequently draws air from the atmosphere, so that its pressure will be in equilibrium with that of the latter. The fall of temperature is also conducted downward and proceeds as a wave, and as it descends it causes a dimunition in volume at the corresponding depths and therefore produces an inward flow of air. This cold wave, however, is preceded by the maximum temperature wave, which as it proceeds downward causes a further expansion of air, which goes to make up for the decreased volume of air caused by the cold wave following immediately after. The difference in temperature, however, of the soil at any depth immediately before and after the maximum temperature wave is reached is very small, as experiments at this Station show; consequently the expansion and expulsion of air caused by the downward march of the minimum temperature wave is not very appreciable. Hence, as the cold wave proceeds downward and produces a decrease in volume of the soil air, the air that comes to make

up for this decrease, so that an equilibrium of pressure will exist, is mainly from the outside atmosphere. After a certain depth is reached, the maximum temperature wave entirely disappears, and there is no more upward expulsion or movement of air. From now on, as the temperature of the soil is further decreased and the volume of its air diminished correspondingly, the current of flow of air into the soil is entirely from the outside atmosphere. This downward flow of air will continue until the soil temperature begins to rise again and the cycle recommences. When the minimum temperature of the surface soil is reached, it is, as a rule, about the same or slightly higher than that of the air immediately above. The temperature of the air at about this period increases with the height in the same manner as the temperature of the soil increases with depth, which is just the opposite from what it is during the day. This increase instead of decrease of temperature at night with a rise in elevation is called "surface temperature inversion." At this minimum temperature the atmospheric pressure approaches its maximum, and the inward flow of air is thereby facilitated.

All the foregoing facts lead to the enunciation of a general law that during the day, as the temperature rises, the soil air tends to flow outward into the atmosphere, and during the night, as the temperature falls, air from the atmosphere tends to flow inward into the soil. This law diametrically opposes the prevalent theory that during the night there is an upward movement of moist warm air. The above law, however, seems to be borne out by logic and appears to be confirmed by experimental evidence subsequently to be presented. The prevalent theory seems unreasonable; for instance, if it is admitted, which it must be, that the soil air escapes into the atmosphere during the day as the temperature rises, then where and when does the soil obtain its air if it continues to give up air even during the night? It might be argued that it is vapor that is rising to the surface and not air. That is inconceivable in the present case. It is true that distillation would occur if the amplitude of temperature were appreciable and constant, but it has been shown that the temperature of the whole column of soil decreases constantly and that an air current from the cold atmosphere is drawn inward which tends to encounter and oppose any upward movement of vapor rising from any difference in temperature. Moreover, granting for sake of argument that there is a vapor rising from the warmer soil to the colder soil at the surface, the amount would be extremely small—too small to account for the great quantity of dew commonly noted—because the temperature amplitudes of the soil at different depths at night are never very great. In fact, during the spring months, as the temperature of the lower depths continually rises and the trend of the air temperature is upward, the range of temperature between the surface and the lower depths, say 6 inches, is small, usually amounting only to about 2° or 3° C. The greatest differences in temperature at the different depths in the morning occur

in the fall, when the trend of the air temperature is downward and the surface soil temperature continually falls. At this time the variation in temperature between the surface and 6 inches of the mineral soils may be as high as 8° C.

The truth of the matter, however, seems to be that instead of vapor rising from the warmer soil below to the colder soil at the surface, vapor enters the soil from the atmosphere. This is a natural conclusion from the law enunciated that during the day air is exhaled from the soil and during the night air is inhaled from the atmosphere. The amount of moisture that will thus enter the soil will depend upon the quantity of air inhaled and upon its absolute humidity, but, as calculations show, it is extremely small. The water may be abstracted by the dry soil at the surface as the air is drawn in or it may enter unaffected. Thus, it is possible that the moisture lost by the soil during the day by the expulsion of its moist air is partly, if not wholly, regained at night.

What is, then, the source of the water of the dew? The greatest part of it comes from the lower layer of the atmosphere itself by condensation. Some of it comes from the leaves of trees and plants; and a certain amount comes from the soil by capillary and thermal capillary action, as set forth previously.

According to the foregoing consideration, therefore, the notion that "dew is formed from the vapor rising from the warmer soil into a colder atmosphere" is wrong, and those who proposed and adhere to this theory seem to be laboring under a misapprehension of facts.

MOVEMENT OF MOISTURE FROM A MOIST AND WARM COLUMN TO A DRY AND COLD COLUMN OF SOIL AND FROM A MOIST AND COLD COLUMN TO A DRY AND WARM COLUMN OF SOIL

The soil moisture under field conditions exists during the warm period of the year nearly always in a gradient form. During a long drought even the upper surface dries out, either of its own accord or induced by artificial means. This layer of dry soil formed at the surface is known as mulch. To this mulch is ascribed the important function of conserving the moisture in the soil by its ability to reduce evaporation of water at the surface. It accomplishes this conservation of moisture, it is claimed, by producing a change or break in the capillary connections between itself and the moist soil below.

Since, on account of the kinetic energy, the absorptive and adhesive forces of the solid substance decrease with a rise in temperature, the interesting question arose whether the dry mulch with an excessively high temperature would absorb moisture from a moist soil with low temperature, even when the capillary connections were ideal. The desire to secure information upon this important and exceedingly interesting point led to the execution of the following experiments: Brass tubes, as described in the preceding sections, were filled with soil, one half with

dry and the other half with moist soil, and the two columns were separated only by a circular piece of cheesecloth, in order to facilitate the separation of the two columns for moisture-movement determinations.

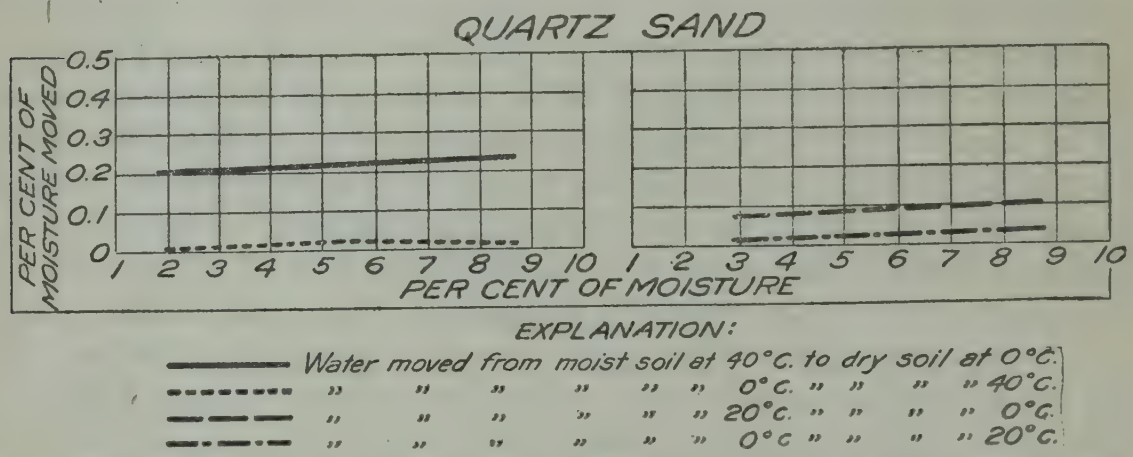
QUARTZ SAND
PER CENT OF MOISTURE MOVED
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
PER CENT OF MOISTURE
EXPLANATION:
Water moved from moist soil at 40°C. to dry soil at 0°C.
" " " " " " 0°C. " " " " 40°C.
" " " " " " 20°C. " " " " 0°C.
" " " " " " 0°C " " " " 20°C.

FIG. 5.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of quartz sand, and from a moist and cold to a dry and warm column of quartz sand.

The tubes were then inserted in the boxes shown in figures 1 and 2, and that portion of the tubes containing the moist soil was kept at 20° and 40°, while that part which held the dry soil was maintained at 0° C.

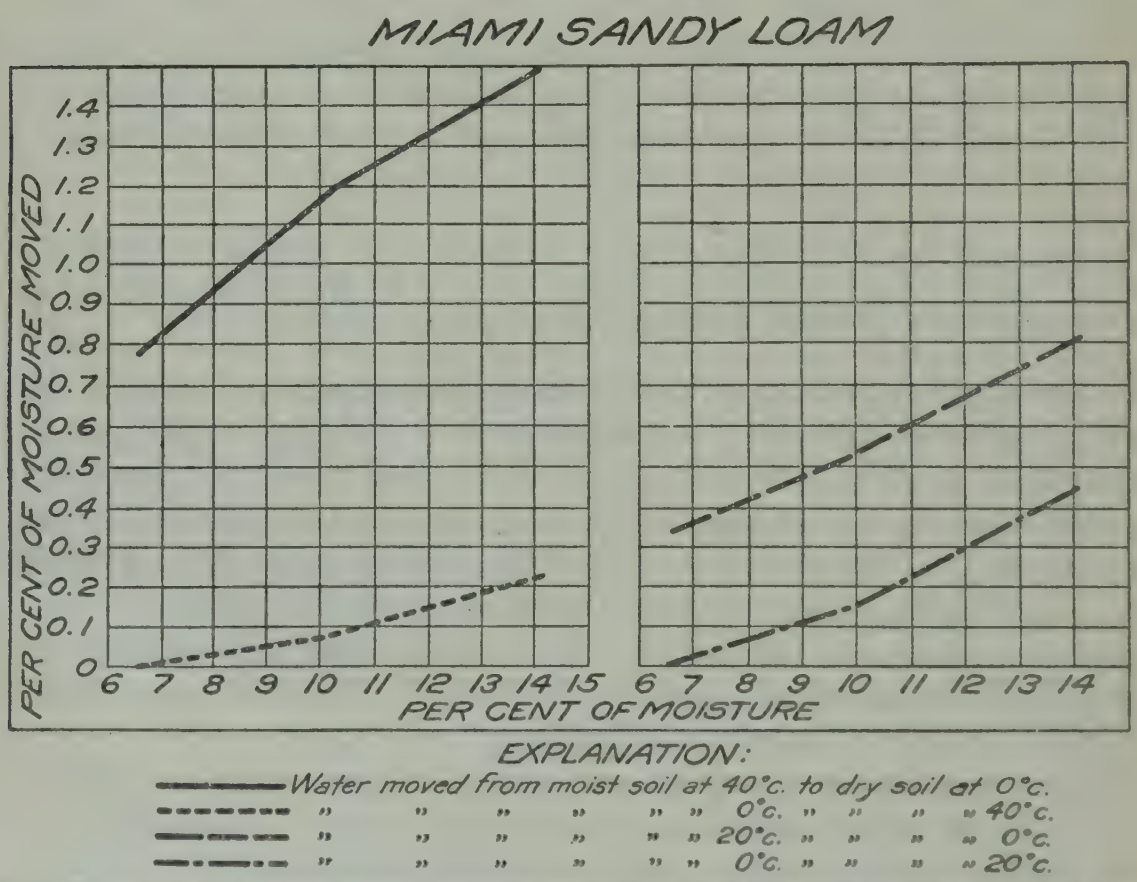

FIG. 6.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami sandy loam, and from a moist and cold to a dry and warm column of Miami sandy loam.

In another set of tubes these temperatures were reversed. The soils employed were the same as those previously described—namely: Quartz sand, light and heavy Miami sandy loam, Miami silt loam, Clyde silt

loam, and Miami clay. There were three different moisture contents used for each soil, designated as low, medium, and high. The duration of all experiments was about eight hours. The numerical data obtained are shown in Table IV. The accompanying figures 5 to 10 represent these same data graphically. Each soil has two charts: The one to the left is for the temperature amplitude of 40°, and the one to the right is for the temperature range of 20° C. The abscissas in every case represent the percentage of moisture content and the ordinates the percentage of water moved either from the moist and warm column to the dry and cold column of soil, or from the moist and cold column to the dry and warm column of soil. The upper curves of each chart represent the

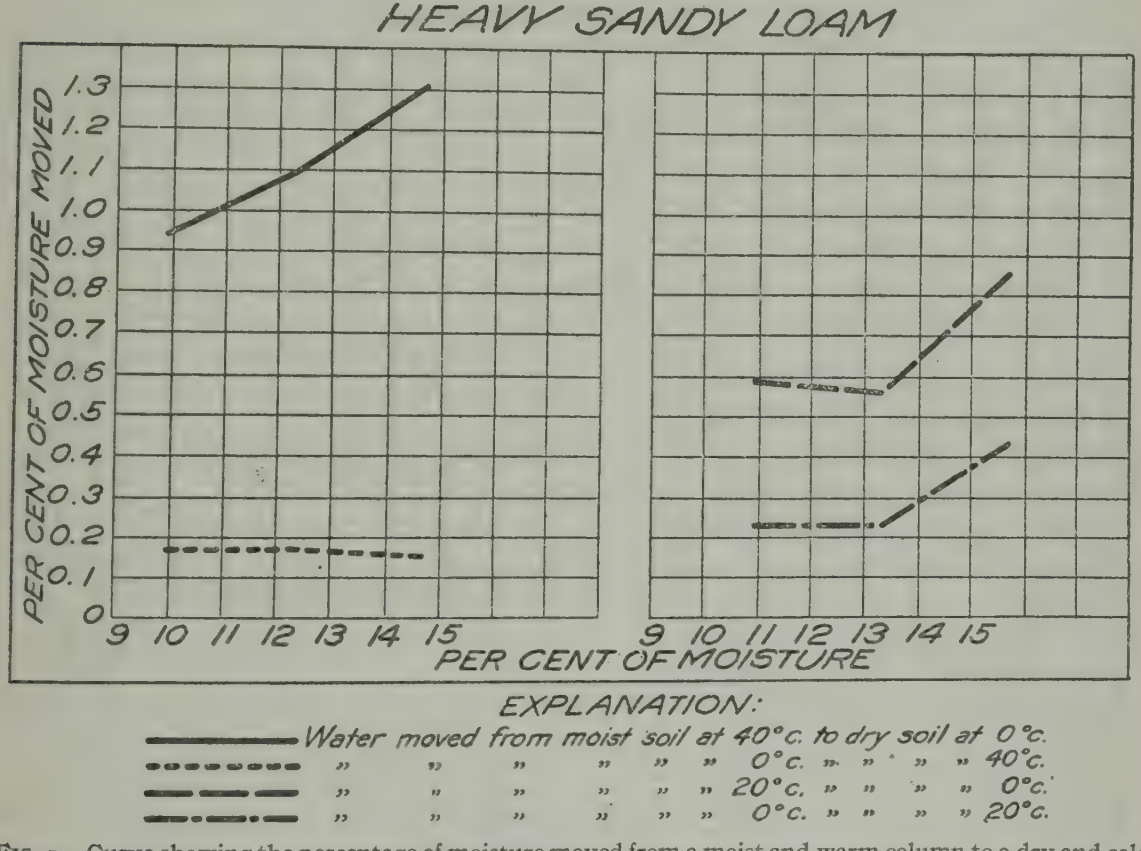

FIG. 7.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of heavy sandy loam, and from a moist and cold to a dry and warm column of heavy sandy loam.

percentage of water movement that took place from the moist and warm soil to the dry and cold soil, while the lower curves show the movement of water that occurred from the moist and cold soil to the dry and warm soil. As in the preceding case, the precentage of moisture moved is based upon the difference in percentages of moisture contained in the dry soil of the beginning and end of the experiment.

Considering first the numerical values showing the amount of water moved from the moist and warm column of soil to the dry and cold column of soil, which are graphically represented by the upper curve of each chart (fig. 5 to 10), it will be seen (1) that this amount is nearly twice as great in the temperature amplitude of 40° as in 20° C., (2) that

it is somewhat greater in soils with higher than with lower colloidal content, and (3) that it increases with the rise in moisture content.

By comparing these results with those obtained with columns of soils of uniform moisture content, some very striking contrasts are revealed. The previous results show, for instance, that the maximum thermal motion of water occurs at a definite but comparatively low moisture content and that the value amounts in some cases to more than 3.50 per cent. The above data show, however, that the maximum movement

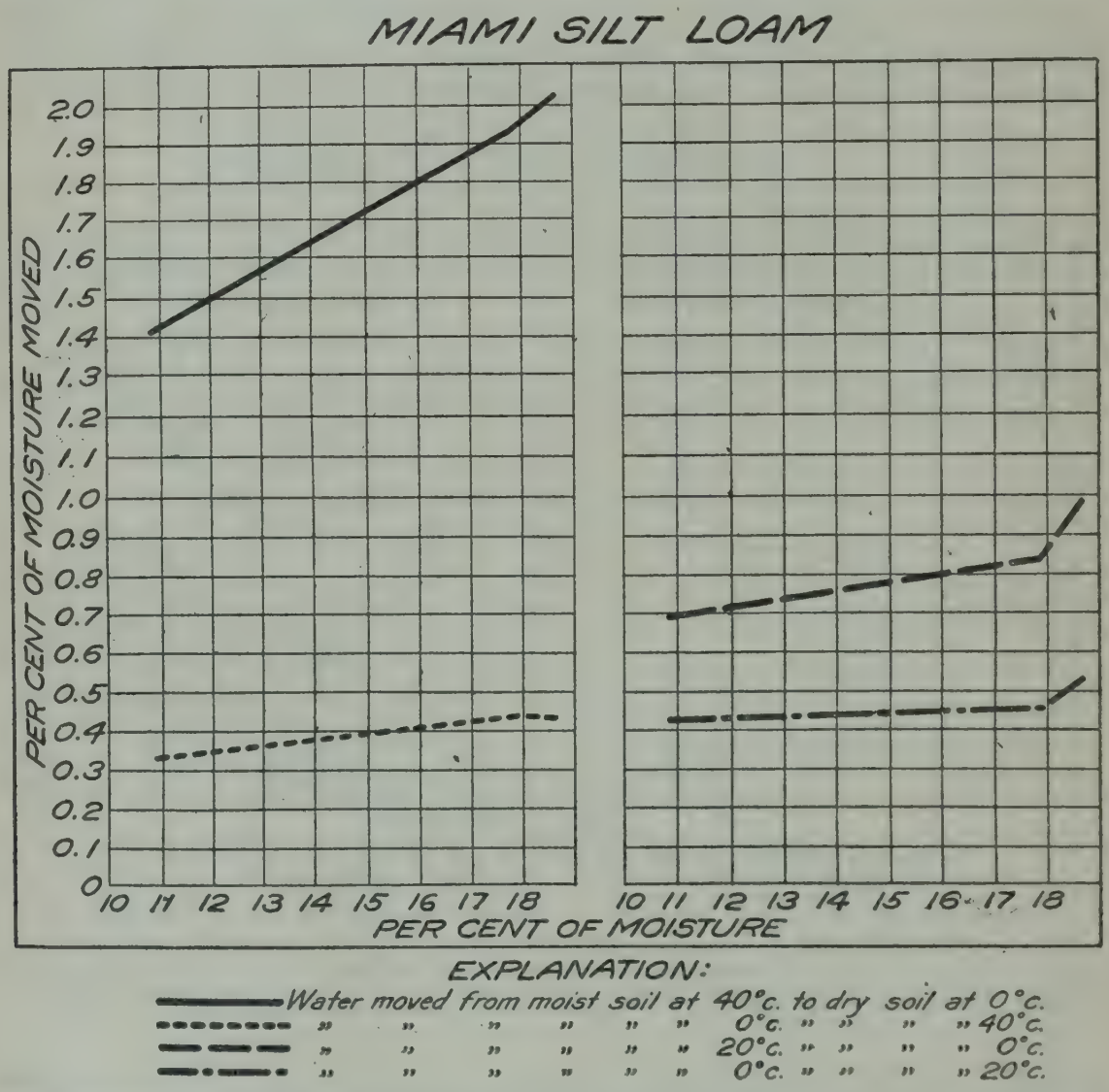

FIG. 8.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami silt loam, and from a moist and cold to a dry and warm column of Miami silt loam.

of water from the moist and warm column to the dry and cold column of soil takes place at the highest water content and that in the majority of cases the percentage of this maximum water translocation is only one-half as great as in the former case.

These apparent differences seem to be easily explainable. The increase of water movement from moist and warm soil to dry and cold soil with a rise in water content is natural and only goes to prove that the water is held by the soil with low moisture content with great force, and consequently it can not be extracted readily and extensively by a greater

abstracting force. When the attractive forces of the soil for water are satisfied and the thickness of the surface and capillary films is increased, then greater quantities of water will be removed by the same abstracting force. The smaller thermal water movement which occurs in the moist and dry soil rather than in the soil of uniform moisture content is due mainly to the cheesecloth which is placed between the dry and moist

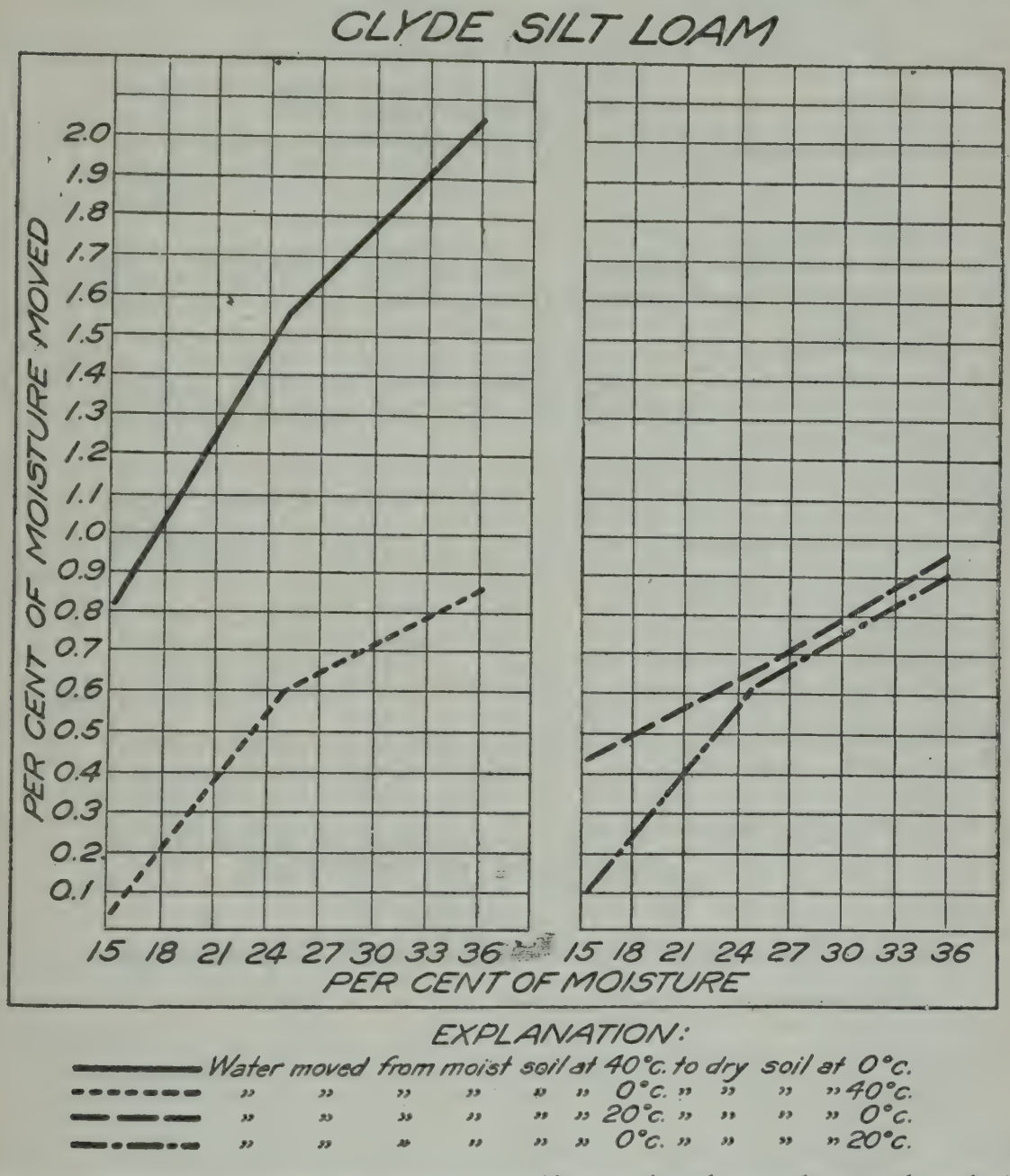

FIG. 9.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Clyde silt loam, and from a moist and cold to a dry and warm column of Clyde silt loam.

columns of soil. Although this cheesecloth was very thin and had wide meshes, yet it prevented the two columns from forming a complete and perfect contact; consequently the dry soil had to absorb water directly through the cheesecloth as well as from the soil.

Another factor which would seem to impede the rate of water movement from a moist and warm to a dry and cold column of soil is the resistance which the dry soils offer to wetting, owing to the air film

surrounding the particles and to any oily substances that might be present. The influence of this factor, however, must be extremely small, if any, because when these soils were slightly damped the amount of water moved was generally less or about the same as before. The common belief that water moves more rapidly in damp than in dry soils is generally exaggerated. When a soil is damped to eliminate the factor of resistance to wetting, its absorptive power for water is decreased correspondingly, so that one factor tends to counterbalance the other, and at the end the

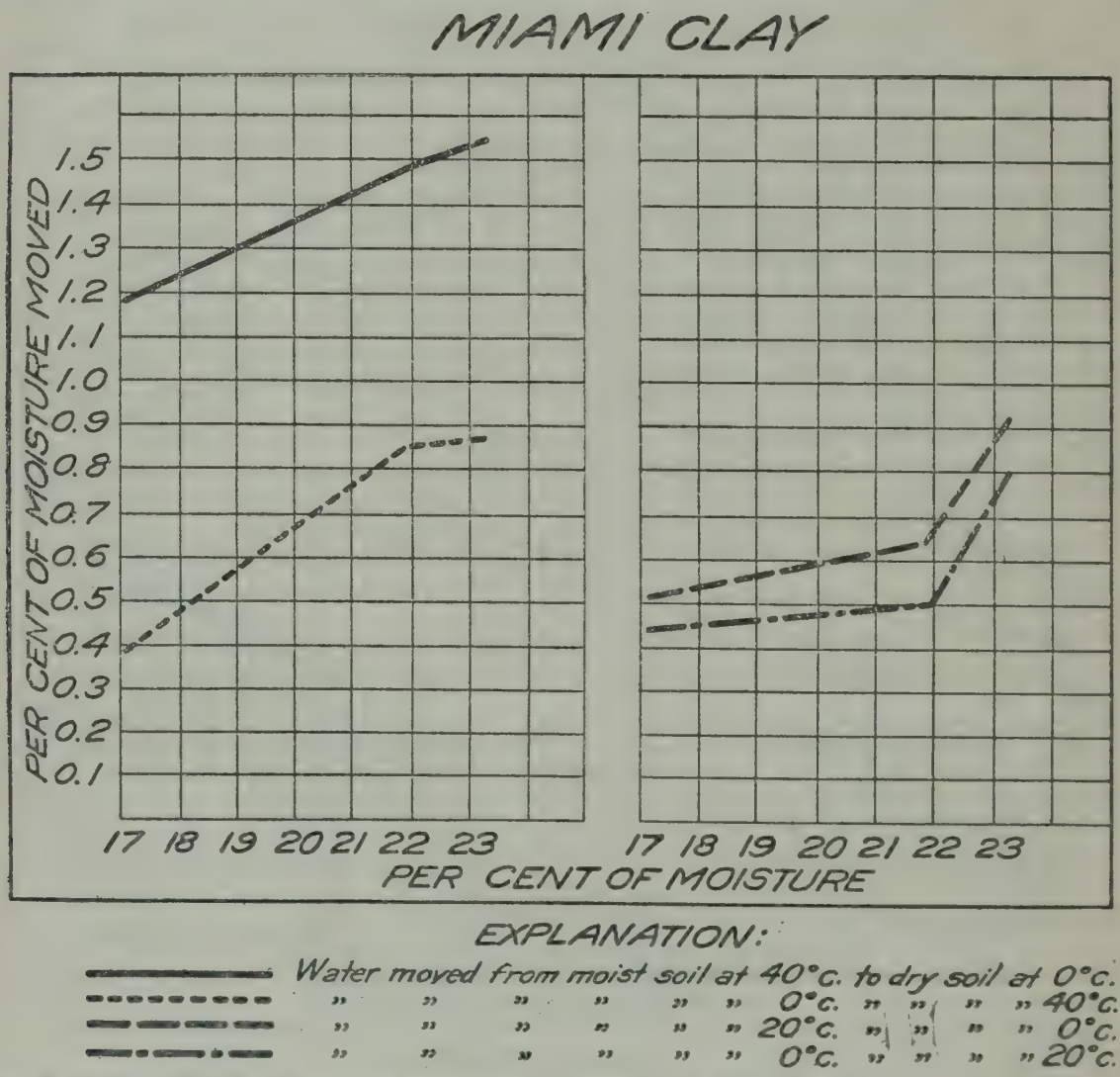

FIG. 10.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami clay, and from a moist and cold to a dry and warm column of Miami clay.

results are about the same. Moreover, the soils which stubbornly resist wetting are not very common.

From the practical standpoint the results of the second part of the present investigation are probably far more important than those of the first part just discussed. These results show the remarkable fact that when the dry soil is kept at 20° and 40° and the moist soil at 0° C., the dry soil takes up very little, if any, water from the moist soil and that this quantity of water absorbed decreases with a rise in temperature. As will be seen from the data, the percentage of moisture absorbed by the dry soil at 20° is in all cases greater than that absorbed at 40° C.

At both amplitudes of temperature the percentage taken up increases with the colloidal content in the soil, which is natural.

TABLE IV.—*Movement of moisture from a moist and warm column of soil to a dry and cold column of soil and from a moist and cold column of soil to a dry and warm column of soil*

Kind and temperature of soil.	Percentage of moisture in soil.		
Quartz sand:			
At beginning of experiment	1.85	5.30	8.75
Movement from moist column at 20° to dry column at 0° C	.0746	.0879	.1129
Movement from moist column at 0° to dry column at 20° C	.0105	.02131	.03912
Movement from moist column at 40° to dry column at 0° C	.2048	.2210	.2376
Movement from moist column at 0° to dry column at 40° C	.0121	.0160	.01522
Light sandy loam:			
At beginning of experiment	6.497	10.141	14.17
Movement from moist column at 20° to dry column at 0° C	.345	.550	.820
Movement from moist column at 0° to dry column at 20° C	.061	.163	.448
Movement from moist column at 40° to dry column at 0° C	.779	1.18	1.496
Movement from moist column at 0° to dry column at 40° C	.000	.08	.235
Heavy sandy loam:			
At beginning of experiment	9.906	12.30	14.695
Movement from moist column at 20° to dry column at 0° C	.592	.569	.863
Movement from moist column at 0° to dry column at 20° C	.215	.211	.445
Movement from moist column at 40° to dry column at 0° C	.937	1.094	1.309
Movement from moist column at 0° to dry column at 40° C	.168	.169	.150
Silt loam:			
At beginning of experiment	10.89	17.88	18.67
Movement from moist column at 20° to dry column at 0° C	.687	.844	.989
Movement from moist column at 0° to dry column at 20° C	.411	.461	.529
Movement from moist column at 40° to dry column at 0° C	1.413	1.942	2.038
Movement from moist column at 0° to dry column at 40° C	.347	.445	.438
Clyde silt loam:			
At beginning of experiment	15.349	25.086	36.18
Movement from moist column at 20° to dry column at 0° C	.429	.662	.962
Movement from moist column at 0° to dry column at 20° C	.100	.606	.900
Movement from moist column at 40° to dry column at 0° C	.814	1.554	2.046
Movement from moist column at 0° to dry column at 40° C	.042	.594	.852

TABLE IV.—*Movement of moisture from a moist and warm column of soil to a dry and cold column of soil and from a moist and cold column of soil to a dry and warm column of soil*—Continued

Kind and temperature of soil.	Percentage of moisture in soil.		
Clay:			
At beginning of experiment	17.05	21.88	23.29
Movement from moist column at 20° to dry column at 0° C.	.514	.653	.923
Movement from moist column at 0° to dry column at 20° C.	.436	.502	.796
Movement from moist column at 40° to dry column at 0° C.	1.180	1.482	1.552
Movement from moist column at 0° to dry column at 40° C.	.380	.850	.873

Obviously, then, the temperature has a tremendous influence upon the absorptive power of soils for water. This is what might be expected from the laws of kinetic energy. According to this law, the energy or motion of the molecules increases with temperature, and consequently the adhesive and absorptive forces of the solid matter for liquids or gases decreases. These results, then, tend to confirm postulate 2 (p. 148), that the attractive forces of the soil for water decrease with a rise in temperature.

The foregoing experimental results and theoretical considerations suggest very strongly that the efficiency of the soil mulches in conserving moisture in the soil is not dependent solely upon their thickness and degree of capillary discontinuity between themselves and the moist soil below, but also upon their temperature. It is well known that the temperature of the surface soils during sun insolation is many degrees higher than that of the air immediately above. In some parts of the world where the sky is clear and the sun insolation very intense, the surface soil may attain a temperature about 40° C. higher than that of the air about 4 feet from the ground. Even at this Station the surface soil temperature of the mineral soils, and especially of the light sandy soils, is very often approximately 15° C. higher than that of the air above. From the surface downward the soil temperature decreases, but in the upper 1 or 2 inches the diminution is far more rapid than at the lower depths, amounting sometimes and in certain soils to more than 11° C. for each inch in depth. When the surface soil is disturbed and a mulch is formed, its heat conductivity is decreased, and the high temperature attained at the surface is not all conducted downward but is compelled to accumulate on the dry mulch and then is radiated back into space. The difference in temperature between the mulch and the moist soil below is sometimes as high as 15° C. at this Station. In arid regions this difference must be far greater.

This excessively greater temperature of the dry mulch diminishes the adhesive and absorptive forces of the dry soil, so that its capacity and intensity to withdraw water from the moist soil below are either entirely prohibited or greatly reduced. The result is that the water is saved from direct evaporation. On the other hand, during the night the soil temperature reverses itself and becomes lowest at the surface and increases with the depth, but the difference between the mulch and moist soil is generally not as great during night as during the sun insolation. Since the attractive and adhesive force of the dry soil and the surface tension of the soil water are increased by the low temperature, the tendency of the soil moisture is to move upward very energetically. To what extent this movement occurs can not be stated with certainty, because the moisture not very far below the mulch is held with a great force and is given up with great reluctancy unless moisture moves from a farther depth below, satisfies the absorptive power, and thickens the surface and capillary films.

Furthermore, the amount of water moved will depend upon the temperature gradient—that is, upon the range of temperature between the surface and lower depths. As already stated, this temperature gradient at night is most marked during the summer and fall and is smallest during the spring. Any water, however, that the mulch pulls up during the night is certain of being evaporated during the day. May it not be, then, that an appreciable amount of water is lost from the soil in this manner?

Temperature not only tends to conserve moisture in the soil after the mulch is formed but also aids and hastens the formation of this mulch. It has been seen that as the temperature of the moist soil at the upper depth increases, the surface tension of the soil water and the adhesive and absorptive forces of the soil decrease. The upward pulling force, therefore, is diminished, and the water is not brought up with sufficient rapidity to keep the upper layers moist, so that a mulch is formed at the top. The diminution of the surface tension of the soil water at or near the surface is very large during the sun insolation and far greater than the increase during the night, because during the sun insolation the soil absorbs heat from the sun very rapidly, and since the soil is a poor conductor of heat the heat is allowed to accumulate at the surface and raise its temperature far above that of the next layers.

The foregoing considerations have been deduced from the experimental data and from the laws of kinetic energy of matter, surface tension of liquids, etc., in their relation to temperature. It is now of great importance as well as of high interest to know whether these deductions can be verified experimentally. The type of experiment which the writer probably would have performed to test out whether or not the temperature does tend to conserve moisture in the soil has

fortunately been performed by Buckingham (5) for another purpose. In his studies on the loss of water under arid and humid conditions, Buckingham endeavored to imitate these two conditions in the laboratory. He placed soil in cylinders 48 inches long and 2½ inches in diameter and provided each cylinder with side tubes at the bottom for the introduction of water. By means of an electric fan he allowed a current of air to be blown over the top surface of the soils. For the arid conditions this current of air was heated without changing its absolute humidity to a temperature of about 50° to 60° F. above the room temperature. To imitate also the high surface temperature of soils under the strong sunshine of arid climates, the top 1½ inches of the cylinders under the hot air was heated by coils surrounding the cylinders to about the same temperature as the hot air. The breeze of about 3 miles per hour was kept going all the time. The heating current was turned on for six

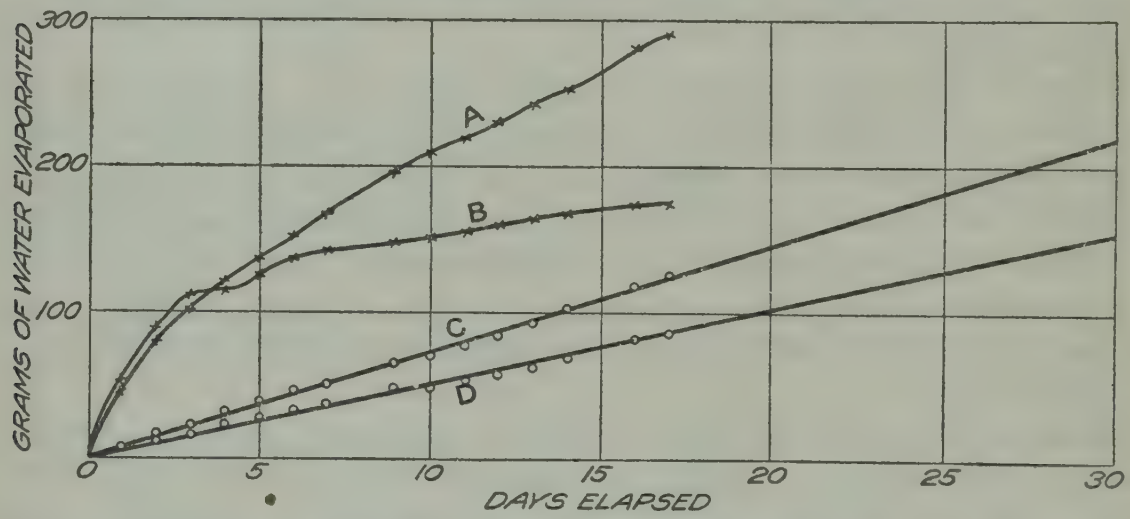

FIG. 11.—Curve showing the evaporation of water from Takoma soil fed with tap water: *A*, Soil under humid conditions; *B*, soil under arid conditions; *C*, water under arid conditions; *D*, water under humid conditions.

hours a day, except on Sundays and holidays. For the humid conditions the soils were placed under the current of air at room temperature. Buckingham performed a number of experiments bearing upon this subject and the results he obtained are qualitatively about the same for all of them. Figure 11 shows a typical set of results.

An examination of figure 11 shows that the loss of water from the soil under arid conditions is much more rapid at first, but after about 4 days have elapsed the rate of loss is less under arid than under humid conditions and continues to be so throughout the duration of the experiment. The rate of evaporation from the soils for the last 10 days is 11.2 inches of rain per year under arid conditions and 51.6 inches of rain per year under humid conditions.

Buckingham explains these results under the supposition that a mulch was formed on the soil kept under arid conditions more rapidly than on the soil kept under humid conditions, and the mulch prevented rapid loss

of water from the former. This explanation is correct, of course, in so far as it represents the result of the mulch, but how this mulch was formed and how it was capable of accomplishing this result he fails to explain correctly. In the opinion of the writer the above results offer an excellent proof that temperature aids and hastens the formation of a mulch, and tends to conserve the soil moisture in the manner previously set forth.

This is a remarkable paradox indeed that a temperature which causes the loss of water should also cause its conservation.

SUMMARY

The main and most important facts presented in the foregoing series of studies may be summarized as follows:

(1) When one half of a column of soil of uniform moisture content is maintained at 20° and 40° and the other half at 0° C. for eight hours the percentage of water moved from the warm to the cold soil increased in all the different types of soil with a rise in moisture content until a certain water content was reached, and then it began to decrease again with further increase in moisture content. The results then plot into a parabola. The percentage of moisture at which the maximum thermal translocation of water occurred is different for the diverse classes of soil, but the percentage of the maximum thermal translocation of water is about the same for all classes of soil for any one of the temperature amplitudes. The percentage of moisture at which this maximum thermal translocation occurred is designated as the "thermal critical moisture content."

These results are contrary to what might be expected from the laws of surface tension and viscosity. They have led to the conclusion that the capillary movement of water in moist soils is not controlled entirely by the curvature of the capillary films, as is generally believed, but also by the unsatisfied attractive forces of the soil for water.

(2) When a moist column of soil was kept at 20° and 40° and a dry column of soil at 0° C. for eight hours and the two columns were separated by an air space, the percentage of moisture distilled over from the moist and warm column to the dry and cold column of soil was very insignificant for both amplitudes of temperature and was about the same for all moisture contents.

These results lead to the conclusion (a) that the amount of water lost from the soil by water vapor is very small; (b) that there is no rising of vapor during the night from the warmer soil below to the cold soil above; and (c) that the water of the dew is not derived from the soil vapor, as is commonly believed.

(3) When a moist column of soil was in contact with a dry column of soil and the former was kept at 20° and 40° and the latter at 0° C. for eight hours the amount of moisture moved from the moist and warm soil

to the dry and cold soil increased with temperature and with moisture content. But when the moist column of soil was maintained at 0° and the dry column of soil at 20° and 40° C. for the same number of hours there was very little, if any, movement of water from the former to the latter.

These results have led to the conclusion that temperature has a very marked influence on the conservation of moisture by mulches.

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SOIL TEMPERATURES AS INFLUENCED BY CULTURAL METHODS

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The data here reported were accumulated under natural field conditions during a period of two years on three plots in a young apple orchard, as follows: (1) Tillage with a cover crop, (2) straw mulch, and (3) grass land. The temperature effect of cultural methods is a detail of a general investigation of the phenomena of orchard soil management. The data have a bearing on other soil problems perhaps important enough to warrant separate presentation at this time.

The temperatures were recorded automatically by means of soil thermographs manufactured by Julien P. Friez & Sons. This instrument consists of a cylinder revolved by an 8-day clock. Blank forms are placed on the cylinder and the temperatures are traced thereon by a pen connected with the thermometer bulb. Temperatures are thus recorded continuously.

The thermometer bulbs were planted 5 or 6 feet northeast of each tree and at a depth of 9 inches. On the straw-mulch plot the bulb was placed under and 12 inches from the outer edge of the mulch collar. Only one instrument was used on each plot. It is felt, however, that the records are trustworthy and portray with reasonable exactness the existing conditions. All instruments were carefully checked with a standard thermometer at the beginning and during the course of the experiment, and their behavior was highly satisfactory. Great care was exercised in changing the chart sheets, to see that each blank was properly placed.

The plots are located on a glaciated, rough, river-bluff, upland soil in southern Indiana. The rocks of the region are limestone, which outcrop on the steeper hillsides. The mechanical analysis shows the soil to be a clay silt. (See Table I.)

TABLE I.—*Mechanical analysis of upper 9 inches of soil on the experimental plots* [a]

Plot.	Fine gravel (2 to 1 mm.).	Coarse sand (1 to 0.5 mm.).	Medium (0.5 to 0.25 mm.).	Fine sand (0.25 to 0.1 mm.).	Very fine sand (0.1 to 0.05 mm.).	Silt (0.05 to 0.005 mm.).	Clay (0.005 to 0 mm.).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
A.....	0.1	0.7	0.8	1.3	1.8	82.1	13.0
C.....	.2	.8	.9	1.4	5.2	78.7	12.5
D.....	.2	.7	.8	1.6	7.3	77.0	12.4

[a] These analyses were made by the Bureau of Soils, United States Department of Agriculture.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 4
Oct. 25, 1915
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Plot A received clean cultivation with a rye cover crop sown in late summer and turned under in the spring. The average depth of plowing has been 7 inches. Cultivation started in 1913 on May 1 and in 1914 on May 11. Rye at the rate of 1½ bushels per acre was sown for a cover crop on August 20 in 1913 and on August 15 in 1914. The land was cultivated seven times each season.

Plot C was in grass, which was cut and allowed to lie where it fell, as in plot D, but in addition a mulch of wheat straw was applied about the same time that plot A was plowed, using one bale to a tree. The bales averaged 93 pounds in weight. The litter was scattered around the trees, forming a collar 12 to 14 feet in diameter.

Plot D was in grass, which was cut and allowed to lie where it fell. In the autumn of 1912 plot D was seeded to a mixture of grasses in which timothy largely predominated and may here be considered as a timothy meadow. The grass was mowed for the first time in the middle of June, 1913, largely to prevent weeds from seeding, as the amount of mulch was negligible. The extremely dry summer of 1914 was disastrous to grass, and a cutting on July 10 returned to the land only one-fifth of a ton of dry hay per acre.

Space does not permit the publication of the complete temperature records, but the weekly maximums and minimums are given in Table II. It will be seen that in April plot A began to forge ahead in holding the highest minimum temperature, with plot D second and C third. This condition prevailed until in the fall, when plot A cooled off quickly and D less quickly, leaving C with the highest minimum temperature until spring. The differences, however, in winter temperatures between the plots were small. During the week of February 23, 1913, plot A showed a minimum temperature of 32° F. and plots C and D, 32.5° F. Plot A continued to cool, until during the week of March 16 it reached 32°, when plot D registered its lowest, 31°, and plot C was 33° F. The following winter the three plots reached their minimum temperatures during the week of January 11, that of plot A being 31°; D, 32.5°; and C, 34° F.

TABLE II.—*Records of soil temperatures under different cultural methods, May, 1913, to May, 1915*

PLOT A: TILLAGE WITH COVER CROP

Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.
	°F.	°F.		°F.	°F.		°F.	°F.
May 5	45.0	58.5	June 16	58.5	68.0	July 28	67.0	76.0
12	53.0	60.0	23	67.0	74.0	Aug. 4	72.0	80.0
19	53.0	63.0	30	67.5	78.0	11	70.0	77.5
26	54.0	63.0	July 7	73.0	80.5	18	71.0	78.0
June 2	54.5	66.5	14	69.0	77.5	25	66.0	77.0
9	60.0	69.0	21	69.0	77.5	Sept. 1	66.0	73.0

TABLE II.—*Records of soil temperatures under different cultural methods, May, 1913, to May, 1915*—Continued

PLOT A: TILLAGE WITH COVER CROP—continued

Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.
	°F.	°F.		°F.	°F.		°F.	°F.
Sept. 8	68.0	76.5	Mar. 30	33.0	50.0	Oct. 19	56.0	60.0
15	59.0	75.0	Apr. 6	41.0	51.0	26	53.0	60.0
22	55.5	66.0	13	38.0	46.0	Nov. 2	45.0	57.5
29	51.0	61.0	20	42.5	55.5	9	45.0	53.0
Oct. 6	55.0	63.0	27	44.0	61.5	16	43.0	52.0
13	54.0	65.0	May 4	51.5	66.0	23	34.0	46.0
20	50.0	59.0	11	52.5	63.0	30	33.0	45.0
27	43.5	51.0	18	52.5	64.0	Dec. 7	43.0	50.5
Nov. 3	.....	.....	25	56.0	67.0	14	37.0	43.0
10	41.0	48.0	June 1	64.0	74.0	21	34.5	37.0
17	37.0	48.5	8	65.5	73.5	28	34.0	35.0
24	45.0	56.0	15	70.0	77.0	Jan. 4	32.0	33.5
Dec. 1	43.0	52.5	22	67.0	74.0	11	31.0	32.0
8	43.0	53.0	29	68.0	79.0	18	31.0	32.0
15	36.0	42.0	July 6	65.0	75.5	25	32.0	33.0
22	36.0	41.0	13	70.0	80.0	Feb. 1	33.0	34.0
29	36.0	37.0	20	68.0	78.0	8	33.0	36.0
Jan. 5	35.0	36.0	27	70.0	80.0	15	32.0	45.0
12	34.0	35.0	Aug. 3	71.0	79.0	22	35.0	40.0
19	33.0	34.0	10	72.0	78.0	Mar. 1	35.0	47.0
26	33.0	37.0	17	70.0	77.5	8	34.0	37.0
Feb. 2	34.0	46.5	24	70.0	80.0	15	35.0	41.0
9	34.0	40.0	31	66.5	74.5	22	36.0	40.0
16	33.0	34.0	Sept. 7	64.0	74.5	29	35.0	41.0
23	32.0	33.0	14	59.5	72.0	Apr. 5	35.0	42.0
Mar. 2	31.0	33.0	21	61.5	73.5	12	40.0	52.5
9	31.0	32.0	28	57.0	74.0	19	44.0	55.0
16	30.0	32.5	Oct. 5	58.0	64.5	26	50.0	66.0
23	31.0	34.0	12	59.0	67.5	May 3	57.0	60.0

PLOT C: STRAW MULCH

May 5	47.0	53.0	Oct. 6	58.0	61.0	Mar. 9	33.0	34.0
12	50.0	53.0	13	57.0	61.5	16	33.0	33.5
19	50.0	56.0	20	51.5	58.5	23	33.0	34.0
26	55.0	57.0	27	50.0	54.0	30	33.0	37.0
June 2	56.0	60.0	Nov. 3	.....	.....	Apr. 6	37.5	42.0
9	58.5	61.5	10	45.0	48.0	13	38.0	43.0
16	57.0	60.0	17	42.0	47.0	20	40.0	48.0
23	60.0	65.0	24	46.0	51.5	27	44.5	49.5
30	64.0	70.0	Dec. 1	47.0	51.0	May 4	49.0	51.5
July 7	68.0	71.0	8	46.5	51.0	11	51.0	52.5
14	67.0	69.0	15	41.0	47.0	18	51.0	54.5
21	68.0	71.0	22	39.5	42.5	25	52.0	55.0
28	66.0	68.5	29	38.0	40.0	June 1	56.0	59.5
Aug. 4	68.0	70.0	Jan. 5	37.5	38.5	8	58.0	62.0
11	66.0	70.0	12	36.0	39.0	15	63.0	65.0
18	70.0	72.0	19	34.0	36.0	22	60.5	64.0
25	68.0	72.0	26	36.0	38.0	29	64.0	67.0
Sept. 1	66.0	69.0	Feb. 2	37.0	41.5	July 6	63.0	65.0
8	66.0	70.0	9	35.0	39.0	13	64.0	67.5
15	62.0	69.0	16	34.0	35.0	20	66.0	68.0
22	61.0	64.5	23	32.5	35.0	27	66.0	69.0
29	57.5	61.0	Mar. 2	33.0	34.0	Aug. 3	65.0	70.0

TABLE II.—*Records of soil temperatures under different cultural methods, May, 1913, to May, 1915*—Continued

PLOT C: STRAW MULCH—continued

Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.	Week ending—	Minimum.	Maximum.
	°F.	°F.		°F.	°F.		°F.	°F.
Aug. 10	65.0	67.5	Nov. 9	51.0	52.5	Feb. 8	35.0	37.0
17	65.0	68.5	16	48.5	52.0	15	35.0	39.0
24	66.0	68.5	23	40.0	50.0	22	36.0	38.5
31	65.0	68.0	30	41.0	45.0	Mar. 1	36.0	40.0
Sept. 7	64.0	67.5	Dec. 7	45.5	47.5	8	36.0	37.0
14	61.5	66.0	14	43.0	46.0	15	36.0	37.0
21	61.5	64.0	21	38.0	43.0	22	37.0	38.0
28	59.0	65.0	28	36.0	38.5	29	36.5	38.0
Oct. 5	58.0	60.0	Jan. 4	35.0	37.0	Apr. 5	35.5	37.0
12	60.0	62.5	11	34.0	36.0	12	38.0	45.0
19	58.0	60.0	18	35.0	36.0	19	42.0	45.0
26	51.0	56.0	25	35.0	36.5	26	45.0	53.0
Nov. 2	56.0	58.0	Feb. 1	35.0	36.0	May 3	52.0	55.0

PLOT D: GRASS LAND

May 5	44.5	57.5	Jan. 5	36.0	37.0	Sept. 7	63.0	70.0
12	48.5	59.0	12	35.5	37.0	14	60.0	67.5
19	49.0	62.0	19	33.5	36.5	21	60.0	66.0
26	53.0	62.5	26	33.0	36.5	28	56.0	66.5
June 2	53.5	66.0	Feb. 2	35.0	44.0	Oct. 5	55.0	60.0
9	58.0	67.0	9	35.0	39.5	12	58.0	62.5
16	57.0	69.0	16	34.0	36.0	19	56.0	58.0
23	66.0	74.0	23	32.5	35.0	26	54.0	56.5
30	65.0	76.5	Mar. 2	32.0	35.0	Nov. 2	46.0	53.0
July 7	70.0	78.5	9	32.0	34.0	9	47.0	51.0
14	67.5	76.0	16	31.0	34.0	16	44.0	50.0
21	.....	.....	23	33.0	34.0	23	37.0	47.0
28	.....	.....	30	32.0	46.0	30	35.0	44.0
Aug. 4	69.0	76.0	Apr. 6	40.0	48.0	Dec. 7	44.0	47.5
11	67.0	74.5	13	37.5	44.0	14	40.0	44.0
18	68.0	74.0	20	41.0	53.0	21	35.5	41.0
25	64.0	73.5	27	44.0	57.0	28	34.5	36.5
Sept. 1	62.5	68.5	May 4	50.5	61.0	Jan. 4	35.0	37.0
8	64.0	69.5	11	52.0	58.5	11	32.5	35.0
15	57.5	69.0	18	53.0	60.5	18	33.0	34.0
22	55.5	62.5	25	54.0	62.0	25	33.5	35.5
29	51.0	57.0	June 1	60.5	67.0	Feb. 1	33.0	35.0
Oct. 6	52.5	59.5	8	60.5	69.0	8	33.0	35.0
13	52.0	60.0	15	65.0	71.0	15	32.5	38.5
20	49.0	56.0	22	63.5	70.0	22	33.0	38.0
27	43.0	47.0	29	67.5	73.5	Mar. 1	35.0	41.0
Nov. 10	41.5	47.0	July 6	63.5	72.0	8	33.0	35.0
17	39.0	49.0	13	68.0	76.0	15	33.5	36.5
24	45.0	54.0	20	67.0	75.0	22	35.0	37.0
Dec. 1	43.5	51.5	27	68.0	77.0	29	35.0	38.0
8	44.0	52.0	Aug. 3	68.0	74.0	Apr. 5	34.0	38.0
15	36.5	44.5	10	69.0	74.0	12	37.0	47.5
27	36.5	41.0	17	67.0	73.5	19	42.5	48.0
29	36.5	37.0	24	68.0	75.5	26	46.0	58.0
			31	65.0	71.0	May 3	53.0	59.0

Plot A maintained the highest maximum temperature during the spring and summer and until quite cold weather in the fall, when plot C registered the highest maximum temperature. This lasted for a month or so during the coldest weather, and as soon as it began to moderate in late winter plot A warmed up rapidly, with D next.

The greatest variation between plots occurred during the summer months. In the spring and fall there is a transition period in which the temperature differences are less. During the summer of 1913 plot A registered a maximum temperature of 80.5° the week of July 7, when plot D was 78.5°, and plot C was 71° F. However, plot C later, the week of August 18, registered a maximum temperature of 72° F. During the week of July 13, the following summer, plot A registered a maximum of 80°; plot D, 77°, the week of July 24; and plot C, 70° F., the week of August 3.

Figures 1 to 4 are reproductions of typical seasonal charts of soil temperatures prevailing under the three cultural systems. These give an idea of the diurnal variations. During the winter the temperatures are quite constant from day to day, with very little variation between plots (fig. 1). In the spring the diurnal range is considerable in the plot under tillage with cover crop and the grass land, but varies little under the straw mulch, which exhibits a very gradual warming up (fig. 2). During the summer season, fluctuations become quite pronounced under tillage and grass, but the straw mulch still maintains its uniformity (fig. 3, *C*). During the season of greatest daily range the maximum and minimum temperatures occur about 10 p. m. and 10 a. m., respectively (fig. 3, *A* and *D*). In the fall the temperatures and ranges are not radically different from those of spring, except that the general trend of temperatures is reversed (fig. 4).

In conclusion, it may be said that a system of clean cultivation with a winter cover crop is characterized by extreme diurnal and annual fluctuations in soil temperature; that a straw mulch equalized these fluctuations to a marked extent, as does also a grass crop, though in less degree.

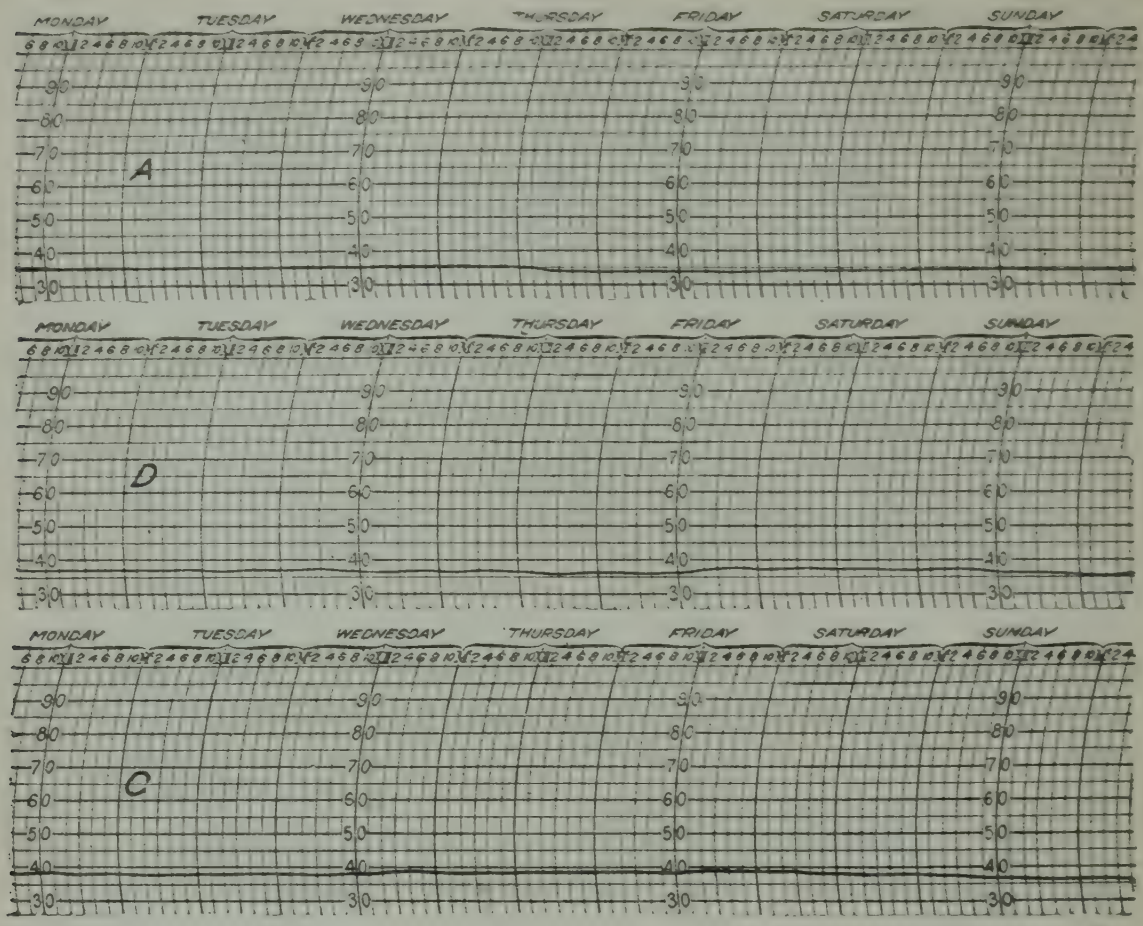

FIG. 1.—Typical charts of soil temperatures during the winter season: Records for week ending January 12, 1914. *A*, Tillage with cover crop; *D*, grass land; *C*, straw mulch.

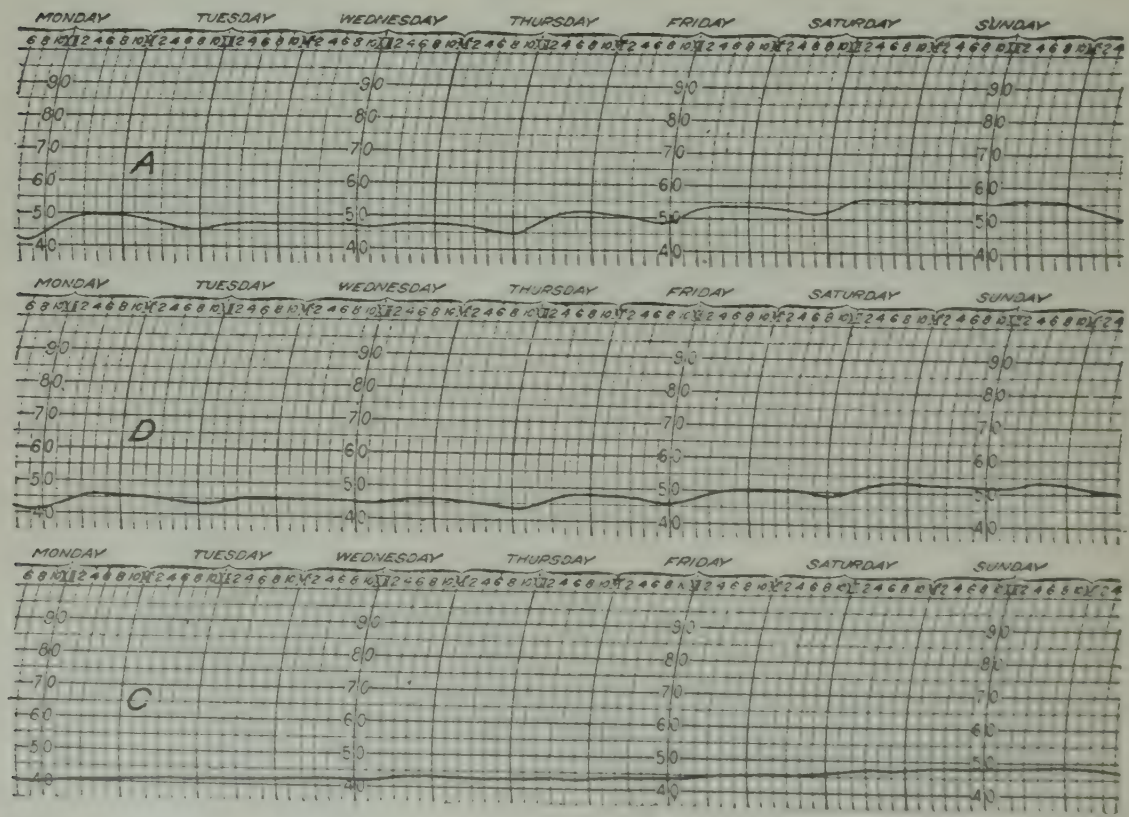

FIG. 2.—Typical charts of soil temperatures during the spring time: Records for week ending April 20, 1914. *A*, Tillage; *D*, grass land; *C*, straw mulch.

FIG. 3.—Typical charts of soil temperatures during the summer months: Records for week ending June 13, 1914. *A*, Tillage; *D*, grass land; *C*, straw mulch.

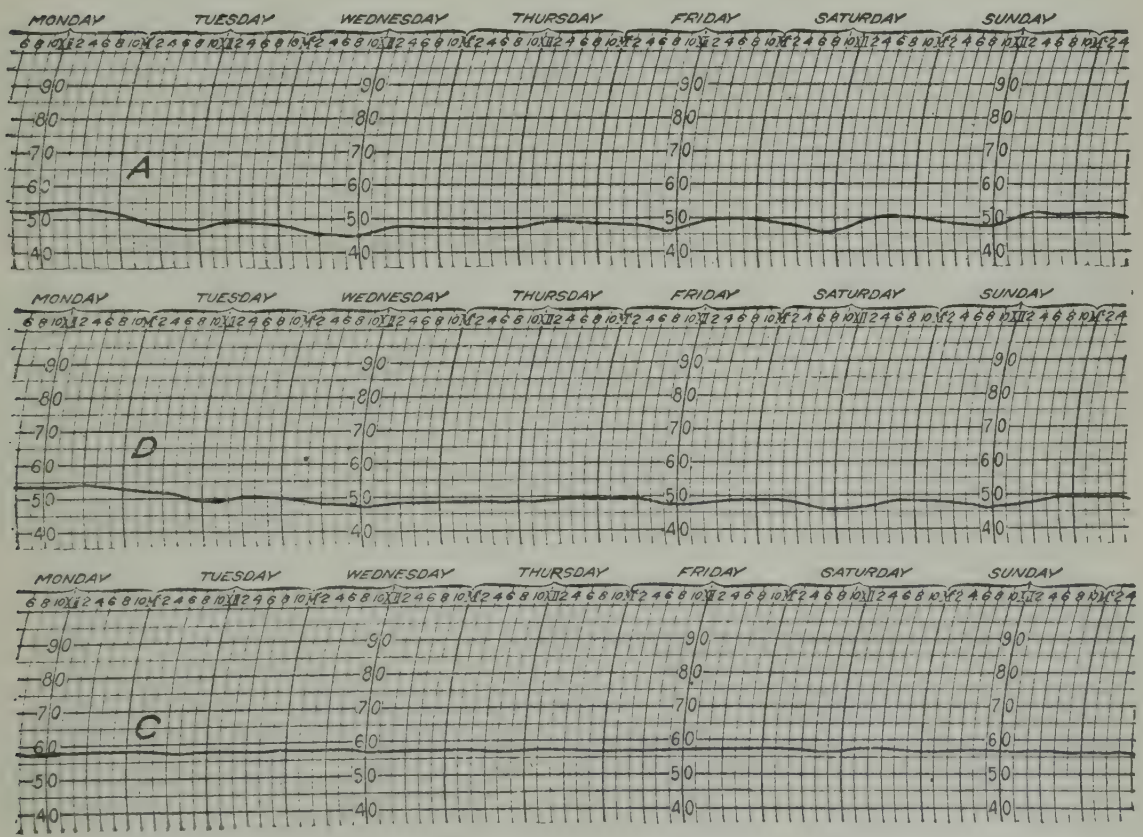

FIG. 4.—Typical charts of soil temperatures during the fall of the year. Records for week ending November 2, 1914. *A*, Tillage with cover crop; *D*, grass land; *C*, straw mulch.

ALTERNARIA PANAX, THE CAUSE OF A ROOT-ROT OF GINSENG

By J. ROSENBAUM, *Specialist in Phytophthora*, and C. L. ZINNSMEISTER, *formerly Agent, Cotton and Truck Disease Investigations, Bureau of Plant Industry*

While working with diseases of ginseng (*Panax quinquefolium*) during the summer of 1913, the authors obtained from a garden near Cleveland, Ohio, roots which showed a peculiar dry-rotted condition about the crown. The dark-brown center of the lesion characterizing this dry-rot was more or less sunken and firm to the touch and gradually shaded into the yellowish white color of the healthy root. It is distinguished from other root-rots by its lack of odor and the fact that the rotted roots never become soft. Plate XII is a reproduction of a photograph of three roots showing the typical lesions of the disease.

When the rot is near the crown of the root, the top of the plant often shows signs of the disease. These signs are a wilting and yellowing of the leaves, which on being disturbed drop off readily at the point of attachment to the main stalk. Such a condition may, however, be caused by other root-rots attacking ginseng, as, for example, the rot caused by *Phytophthora cactorum*.

Because of the unusual character of these lesions, numerous isolations were made from them, and in all cases an Alternaria-like fungus closely resembling *Alternaria panax* Whet. was secured in pure culture. In order to determine whether these two fungi were identical, a series of inoculations on roots and tops were made with both cultures. In addition, a study was made of their macroscopic and microscopic appearance. This work was begun during the summer of 1913 in Ohio and repeated during the summer of 1914 in New York.

In the main two methods of inoculation were followed. Healthy roots were taken from the garden, washed, freed from their fiber roots, sterilized for 10 minutes in a 1 to 1,000 solution of mercuric chlorid, washed in sterile distilled water, and placed in sterilized test tubes. The roots were then injured by making an incision in them with a sterile scalpel, and in this incision was placed a small portion of the fungus from a pure culture. Roots treated in the same way but not inoculated were used as checks. Six series of inoculations were made in this manner, using the Alternaria-like fungus isolated from dry-rotted roots. Ninety-five per cent of infection was secured, and the checks in all cases remained healthy. Typical lesions (Pl. XII) were produced in every instance. In no case did the rotted condition involve the entire root. The time necessary after inoculation for the lesion to appear varied from seven to nine days. Once established the progress of the rot was also very slow.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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At the time the above series were being run, five series of similar inoculations were made with a pure culture of *Alternaria panax*, the necessary checks for each series being used. One hundred per cent of infection was obtained with this fungus, the symptoms and lesions resulting from the inoculation being in every case similar to and indistinguishable from those obtained with the Alternaria-like fungus. Plate XIII, figure 1, shows a longitudinal section through one inoculated root.

In order to test further the pathogenicity of these fungi and to confirm their identity, inoculations were made directly in the soil on roots to which the tops were still attached. Six series were made with the Alternaria-like fungus and five with *Alternaria panax*. The soil was removed from around the crown of the roots and an incision was made in the crown. Into this incision was placed the inoculating material from pure cultures of the two fungi. Ninety-two per cent of infection resulted from the Alternaria-like fungus and eighty-five per cent from *Alternaria panax*. The symptoms and lesions were again characteristic and similar in each case.

Further inoculations were made on the tops by inoculating the leaves with mycelium from pure cultures of both fungi. For some unexplainable reason, or owing to the plants having been sprayed with Bordeaux mixture, no definite results were secured during the summer of 1913. In June, 1914, the work was repeated. Typical leaf-spots of *Alternaria panax* were produced in abundance with both fungi. Plate XIII, figure 2, is a reproduction of a photograph of the lesions produced on ginseng leaves with the species of Alternaria isolated from roots. Spores from these spots were secured and examined. No differences could be noted.

Reisolations were made from the inoculated roots and leaves, and a fungus identical with the original one used for inoculating was obtained.

Numerous attempts to produce infection on the roots without previously injuring them gave only negative results.

Inasmuch as these fungi show no cultural differences and as both are able to infect the leaves and roots of the ginseng plant, the only conclusion warranted by the data at our disposal is that they are identical. This being the case, the blight problem confronting the ginseng grower becomes more complicated. Heretofore it has not been supposed that *Alternaria panax* is able to cause a rot of the root.

The above facts warrant the ginseng grower in taking other means besides spraying in the control of this disease. The means recommended, in addition to spraying, are (1) care in transplanting so as to injure the roots as little as possible, (2) the removal of all tops and stems in the fall, and (3) where the crowns of the roots are sufficiently deep below the surface of the soil, burning over the surface of the bed with a thin layer of straw after the tops have been removed.

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JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., NOVEMBER 1, 1915 NO. 5

SOME POTATO TUBER-ROTS CAUSED BY SPECIES OF FUSARIUM

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INTRODUCTION

Deterioration of tubers of the Irish potato (*Solanum tuberosum*) is induced by a variety of causes. Economically the most important of these are the organisms *Phytophthora infestans*, *Fusarium* spp., bacteria, and miscellaneous fungi, including *Rhizopus nigricans*.

Phytophthora infestans, which is somewhat restricted to the northeastern part of the country, does more or less damage each year, and occasionally in epidemic form causes tremendous losses. Exclusive of *P. infestans*, however, species of Fusarium are undoubtedly the most important causes of tuber decay. Though never occurring in epidemic form with losses comparable to those of late-blight, they are present wherever potatoes are grown, taking their quota of the crop both in the field and in storage.

Several species of the genus Fusarium Link have been described as causes of tuber-rots of *Solanum tuberosum* (Clinton, 3; Pizzigoni, 12; Wehmer, 15; Smith and Swingle, 14; Pethybridge and Bowers, 11; Longman, 6; Manns, 7).[2] In most cases prior to 1912 *F. solani* (Mart.) Sacc. or some species thought to be a synonym of it is given as the causal organism. Until recently the chaotic condition of the genus Fusarium has precluded careful work with clearly defined species.

[1] Having been associated with Dr. H. W. Wollenweber, of the Bureau of Plant Industry, during the past two years, the writer has enjoyed the privilege of personal work with the species and strains cultivated during this period in connection with his monographic study of the genus Fusarium. Any attempt to work with the species of this form genus emphasizes the necessity of completing such studies. Owing to Dr. Wollenweber's absence during the preparation and publication of this paper, he is not responsible for the subject matter. It is regretted that his criticism of the results is lacking, particularly as the data obtained force the author to conclusions which differ somewhat from Dr. Wollenweber's published opinions.

[2] Reference is made by number to "Literature cited," pp. 208–209.

For a list of the more important references to potato studies, see the following: Appel, Otto. Beiträge zur Kenntnis der Kartoffelpflanze und ihre Krankheiten. I. *In* Arb. K. Biol. Anst. Land u. Forstw., Bd. 5, Heft 7, p. 415–435. 1907.

Conclusive work on species of Fusarium which produce tuber-rot with sufficiently delimited species dates from Appel and Wollenweber's fundamental work on the form genus Fusarium. During the progress of these studies Wollenweber established the wound parasitic nature of *Fusarium coeruleum* (Lib.) Sacc. and *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw., and the causal relation of these species to a definite type of rot. Jamieson and Wollenweber in 1912 (5) described an external dry-rot caused by *F. trichothecioides* Wollenw. Wollenweber in 1913 (19, 20) extended the list of species of Fusarium causing tuber-rot by the addition of the following: *F. ventricosum* App. and Wollenw., 1910, and *F. rubiginosum* App. and Wollenw., 1910 [considered a synonym of *F. culmorum* W. G. Sm., 1884, by Wollenweber, 1914 (21)]; *F. subulatum* App. and Wollenw., 1910, as a weak wound parasite under special conditions; *F. orthoceras* App. and Wollenw., 1910, and *F. gibbosum* App. and Wollenw., 1910, as probable causes of tuber-rot.

Jamieson and Wollenweber's description (5) of the powdery dry-rot caused by *F. trichothecioides* is the first description of a definite rot conclusively demonstrated to be caused by a species of Fusarium which is sufficiently described in its normal [1] stages to insure certain identification. However, Wilcox, Link, and Pool (17) published a description one year later of the same disease and subnormal stages of the same organism, for which they proposed a new name—i. e., *F. tuberivorum* Wilcox and Link. The examination of material similar to that used by Wilcox and Link from Alliance, Nebr., demonstrated that *F. tuberivorum* is identical with *F. trichothecioides*.

The increasing number of rotting tubers submitted to the Department indicated the existence of several types of a rot not hitherto described which were caused by species of Fusarium and focused the author's attention during the past year on a laboratory study of these diseases. The object of this paper is to demonstrate the parasitic nature of certain species of Fusarium and to contrast these organisms and the resulting types of deterioration with those already recognized. The economic importance of these rots and the interest manifested by pathologists in a general group of diseases caused by species of Fusarium suggested the advisability of a comprehensive treatment of the species known to cause decay as an aid to their diagnoses and ultimate control.

The tuber-rots considered in this investigation are all of the stem-end and wound-parasitic type. They are not sharply differentiated from each other nor from those previously described as caused by the following species: *F. coeruleum; F. discolor*, var. *sulphureum; F. trichothecioides*. After having made isolations from several hundred submitted specimens of stem-end-diseased tubers and from many more rotting as the result of wound and lenticel invasion or inoculation with known species, the

[1] For a discussion of the idea "normal" as used in this paper, see Wollenweber (21, p. 255–257).

author is convinced that in many cases the only sure way to determine the cause is by cultural studies. In general, specimens of the types of rot developed spontaneously in the field or storage are more characteristic than those produced by inoculation and developed under uniform conditions.

The powdery dry-rot with pink-mycelium-lined cavities caused by *F. trichothecioides* is quite characteristic and not easily confused with the others; the same is true of the rot produced by *F. discolor*, var. *sulphureum*, with its ocherous yellow mycelium, but the rot caused by *F. coeruleum*, in its typical form with external dark-blue mycelium masses and internal blue coloration of the tissues, may be easily confused with some of those herein described unless mature spores are found on the specimen or high cultures are obtained. On some tubers more than one of the wound-parasitic types of Fusarium are present; in others, the diagnosis is complicated by the secondary action of bacterial and fungous saprophytes. While the author can in typical cases determine the cause of Fusarium rot without the preparation of cultures, the latter is not infrequently the safer method. Our inability to differentiate surely the various rots macroscopically complicates the attempt to differentiate them as types caused by specific organisms.

METHOD OF TESTING PARASITISM

The method employed to demonstrate the wound-parasitic nature of species of Fusarium will be outlined in detail before proceeding with the discussion of the several types of tuber-rot and the inoculations with the causal organisms.

Sound tubers as free from skin diseases as possible were selected from the following varieties of potatoes: Burbank, Netted Gem, Early Rose, Idaho Rural, Jersey Peachblow, People's, and Pearl grown at Jerome, Idaho, in 1913 and 1914 and each year kept in cold storage at Washington, D. C., until needed; Irish Cobbler grown in Maine in 1913 and kept in storage through the winter; Green Mountain grown at Arlington, Va., in 1914 and used soon after harvesting.

The selected tubers were washed and disinfected in a solution of 0.5 per cent of formalin, in the majority of the experiments for half an hour, and rinsed in distilled water. Some tubers taken at random were wounded with a large platinum needle, dipped in distilled water, immediately wrapped in waxed paper, and placed in disinfected Altmann incubators. Other tubers were similarly wounded, dipped in distilled-water spore suspensions of the organism to be tested, wrapped, and placed with the controls.

By this method there are chances for secondary invaders, but the used organism is primarily the predominating one. In addition to the control tubers, in every case reisolation, identification in pure culture, and

reinoculation were depended upon to check the work. In many cases transfers of the original strains or of the reisolated ones, or of both, and of any intruders were made to raw, sterile cut potato blocks.

The identification of the closely related species of Fusarium employed in this work involved the careful preparation, purification, and morphological study of high cultures. The nutrient media found of most value in obtaining such cultures are as follows: Potato cylinders, rice, stems of cotton (*Gossypium* spp.), and sweet clover (*Melilotus alba*). Agar media were never used, except for plating. As emphasized by Dr. Wollenweber, the vegetable media are very valuable for encouraging characteristic development of species of Fusarium.

The control tubers were carefully examined for rot about the wounds. These tubers usually remained as sound as when placed in the incubator, only 4 out of some 140 used as controls having any rot whatever. Sprouting of the inoculated tubers and controls demonstrated their continued viability.

Throughout the incubation periods a maximum humidity was maintained, and necessarily the ventilation was bad. Readings of the temperatures were taken twice daily, and this factor is indicated by the average of all readings obtained from the particular compartment during the stated period. The temperatures were not constant, varying a degree or two above and below the average, but the average as recorded represents very nearly the actual storage temperature, since such fluctuations as occurred were of a temporary nature.

It may be considered by some pathologists that the method is an extreme one that under the given conditions any organism might be expected to cause a rot. It is believed, however, that the conditions maintained are no more extreme than those to which potato tubers are frequently subjected in field and storage. The following facts tend to establish the validity of the method: (1) Certain organisms—for example, *F. moniliforme* Sheldon, *F. martii* (*sensu strict.*), *Verticillium albo-atrum* Reinke and Berthold, and *Sporotrichum flavissimum* Link—did not cause a rot under these conditions (see p. 201). (2) *F. solani*, *F. vasinfectum*, a species of Mucor, and one of Rhizoctonia were doubtfully wound-parasitic (see p. 192). (3) The wounded controls remained sound except in a few cases where they were in contact with badly rotted tubers; the same organism was isolated from such controls as from the inoculated tubers in the same compartment. (4) The species of Fusarium herein reported as wound parasites grow and rot sterile cut potato blocks in pure culture; none of the intruding organisms (bacteria or fungi) were able to do this, except that in a few cases the submerged part of the block was attacked. These facts, in addition to the experiments, seem to warrant the conclusions reached.

Since the tubers inoculated with the several species of Fusarium were treated uniformly and the rots developed by the respective species were

much alike, detailed accounts of the appearances presented are of doubtful value and are eliminated. With every rot-producing species of Fusarium included in the experiments the effect was essentially the same—at minimum temperatures, a slow dry-rot; at maximum, a very wet rot, with the tubers completely softened in two or three weeks. Sometimes in the former a mycelium-lined cavity is developed, surrounded by a zone of tissue appearing water-soaked—i. e., a zone of enzymic activity; in other tubers at higher temperatures the same organism proceeds to soften the tuber in a stratiform manner, the several layers reaching across the tuber. Bad-smelling rots did not occur with the species of Fusarium. Such rots associated with *Fusarium* spp. were found to be mixed infections. When *Fusarium* spp. *per se* rot potatoes, an odor suggesting ammonia and trimethylamin is developed.

Rots caused by species of Fusarium are commonly spoken of as either "dry-rots" or "wet-rots." The former are a result of comparatively slow development at low temperatures. The experiments show that any of these organisms capable of causing a rot work more rapidly in an environment of optimum temperature accompanied by high humidity, the tubers developing a wet-rot (see p. 196). Upon drying out, the condition would be termed a "dry-rot." The two forms grade insensibly into each other, so that neither term is specific. The examination of potato tissues rapidly softening as a result of inoculation with pure cultures of *Fusarium* spp. indicates that the middle lamella is dissolved considerably in advance of the fungus; the hyphæ ramify between the cells, but do not appear to enter them at once. Ultimately the contents of the cells are liberated, and the starch grains become more or less corroded.

It should be noted that the experimental data, revised and grouped under the respective organisms, were obtained through a series of experiments covering a period of more than a year. For example, the data on *F. oxysporum* (see p. 191) were extracted from eight different experiments which included several other species and show at a glance the comparative effect of original and reisolated strains on different varieties of potatoes at sundry temperatures.

In the notes on the artificial inoculations recorded under the respective organisms the history of the various strains is first outlined, followed by a brief consideration of the results in text and tabular form.

CERTAIN FIELD AND STORAGE ROTS OF POTATO TUBERS AND THEIR CAUSE

TUBER-ROT CAUSED BY FUSARIUM OXYSPORUM AND FUSARIUM HYPEROXYSPORUM

In a study of a wilt and dry-rot of *Solanum tuberosum*, Smith and Swingle (14) attributed both manifestations to a species of Fusarium. After a consideration of the incomplete nature of previous descriptions

of species of Fusarium occurring on the potato, they chose the name of the earliest one for their fungus—i. e., *F. oxysporum* Schlechtendahl, 1824. This species was not differentiated from *F. solani* (Mart.) Sacc. and other species occurring on potatoes; although no inoculations are recorded by Smith and Swingle, *F. oxysporum* has been generally accepted as the cause of both the wilt and the dry-rot.

Manns (7) made inoculations with a species of Fusarium isolated from the blackened vascular ring and one from dry-rotting tubers, confirming the work of Smith and Swingle (14). He writes as follows (7, p. 316): "In the infection work both of the organisms were wilt producing, bringing about symptoms quite typical with that of the Fusarium blight in the field." Tuber-rot as a result of inoculation with a pure culture of his *Fusarium* sp. is not recorded. Like Smith and Swingle (14), he did not consider *F. oxysporum* different from *F. solani.*

Wollenweber (19, 20), after a study of *F. oxysporum* obtained from the vascular system of vines and tubers, was convinced that this species causes the wilt and stem-end ring discoloration, but not a tuber-rot. It simply winters over in the stem end of the tubers. A few quotations show his view regarding this species of Fusarium:

> * * * the fungus [*F. oxysporum*], a typical xylem inhabitant does not entirely destroy the tuber without the help of tuber rot Fusaria or bacteria [20, p. 42].
>
> The fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for overwintering, without producing a rot of the parenchyma, leads to interesting comparisons * * * [20, p. 42].

Referring to this fungus in his diagnosis, he states that it is a "* * * vascular parasite, cause of wilt disease, but not tuber rot, of *Solanum tuberosum*" (20, p. 28).

To facilitate the arrangement of the species, Wollenweber (19, p. 32) established six provisional sections of the genus Fusarium based upon physiological and morphological characters. One of these sections, Elegans, comprises the vascular parasites, including *F. oxysporum.*

In general, Wollenweber's views in regard to *F. oxysporum* as indicated above are supported by the writer, but the experience of the last year indicates that these statements should be somewhat modified. The repeated isolation of *F. oxysporum* and related forms of the section Elegans from tubers rotting in field and storage, accompanied by the failure in many such cases to obtain any other organisms capable of producing a rot, indicates something more in the nature of this organism than passive hibernation in the vascular ducts of the stem end of potatoes. That the latter may be the chief rôle of the strain of *F. oxysporum* which causes wilt is not doubted. But there are strains of *F. oxysporum* and related forms present in stem-end ring disease and dry-rot which entirely destroy[1] the tubers under the experimental conditions outlined

[1] The fact that *F. oxysporum* is capable of destroying potato tubers is confirmed by Dr. Lon A. Hawkins, of the Bureau of Plant Industry, in unpublished studies on the chemistry of rots of *Fusarium* spp. He employed *F. oxysporum* 3395, a reisolation of strain 2413 (see p. 190).

in another part of this paper. This statement is based upon the results of inoculation work with several strains of *F. oxysporum* isolated from various sources and includes two identified by Wollenweber—i. e., Nos. 1948 and 2413. (See p. 190 and Pl. XV, fig. 3.) The following species and varieties of the section Elegans were found to produce tuber-rot in varying degrees: (1) *F. oxysporum.* (2) A related form which differs by producing an abundant pionnotes on potato cylinders. (See p. 206 and Pl. XV, fig. 1, 2.). Morphologically this fungus is identical with *F. hyperoxysporum* (21, p. 268), described as a cause of stem-rot of the sweet potato (*Ipomoea batatas*) by Harter and Field (4, p. 287, 291). The experiments thus far carried out indicate its biological identity—i. e., *F. hyperoxysporum* isolated from *Ipomoea batatas* caused a similar rot under the same conditions. (See p. 192.) (3) *F. vasinfectum* Atkinson, the cause of cotton wilt. (4) Its homologue isolated from wilt of okra (*Abelmoschus esculentus*). The numerous forms of the section Elegans type, many of which appear to be morphologically identical but biologically different, require further study, and it is not proposed to enter into a taxonomic consideration of these forms at this time. (See p. 206.)

It seems probable that *F. oxysporum* is incapable of readily penetrating the wall of the xylem. When it enters the vascular ring of the tuber from the wilting mother plant, it hibernates therein during the resting period of the tuber and enters the sprouts with the renewal of vegetative activity. At other times as a wound or lenticel invader, plenty of suitable nourishment is at hand, and it produces a dry-rot or a wet-rot, according to the conditions of temperature and humidity. Possibly as a wound parasite it is without incentive or opportunity to enter the vascular ducts.

Although Smith and Swingle (14) and Manns (7) did not differentiate their *F. oxysporum* form *F. solani* and other species occurring on potato tubers, no evidence has been deduced to show that they were not in the main dealing with the effects of a single species or to prove that *F. oxysporum* does not cause a tuber-rot.

Further notes on *F. oxysporum* as a cause of tuber-rot are given under "Jelly-end rot" and in the experiments.

INOCULATION OF POTATO TUBERS WITH FUSARIUM OXYSPORUM, FUSARIUM HYPEROXYSPORUM, AND FUSARIUM VASINFECTUM

FUSARIUM OXYSPORUM Schlecht.—*F. oxysporum* 2997; isolated on March 10, 1914, from a tuber affected with stem-end ring disease and vascular necrosis, from Everest, Kan. Culture used, 16-day-old pionnotes on stem of *Melilotus alba.* As indicated in Table I, all tubers of the four varieties Jersey Peachblow, Idaho Rural, Early Rose, and People's were rotting after 19 days' incubation at an average temperature of 23.1° C. (See Pl. XV, fig. 3.) The least affected variety

was Idaho Rural. However, many of these were almost completely destroyed, being very mushy and "leaky."[1] The organism was recovered from all varieties, two reisolations being made from the Rurals.

F. oxysporum 2999; isolated on March 14, 1914, from a tuber with wound-invading brownish dry-rot from Brookings, S. Dak. Culture, 16-day-old pionnotes on stem of *Melilotus alba*. The results were the same as with strain 2997. The organism was recovered in all attempts, reisolations being made from all varieties except Early Rose.

F. oxysporum 3045; a reisolation of strain 2997 from a rotted tuber of the Idaho Rural variety 20 days after inoculation at 23.1° C. After incubating for 21 days at an average temperature of 25.6° C. all tubers of all varieties—i. e., Netted Gem, Idaho Rural, and People's—showed a deep, progressive rot, a brown zone about the inoculation prick surrounded by a water-soaked area more or less brown in color. The organism was recovered by three isolations.

In a subsequent trial with strain 3045, inoculating the four varieties Idaho Rural, Netted Gem, Burbank, and Pearl with a 1-month-old culture on a stem of *Melilotus alba* and incubating for 37 days at an average temperature of 20.4°, similar results were obtained. Seven reisolations were identified from this lot.

F. oxysporum 1948; isolated and identified by Dr. Wollenweber from a secondary rot following infection by *Phytophthora infestans*. Material from Honeoye Falls, N. Y., February, 1913. Culture used was 1 month old on stem of *Melilotus alba*. The results at different incubation periods and temperatures are as follows: Ten tubers incubated for 24 days at an average temperature of 24.6° rotted, four slightly decaying in all punctures and six wet-rotting. Organism recovered. One tuber at 18.4° rotted in 51 days, while one at 17.8° failed to decay in this time, but the organism persisted.

F. oxysporum 2413; isolated and identified by Wollenweber in January, 1913, from a potato of the Up-to-Date variety, grown on Potomac Flats, Washington, D. C., in 1912, affected with the ring disease. Cultures used, one on stem of *Melilotus alba* and one on a potato cylinder 25 days old. Result of incubation at 25.7° C. for 14 days: All inoculated tubers decayed, 50 per cent being very badly decomposed with wet-rot; organism recovered by four reisolations. Two tubers incubated at 17.8° and 18.4°, respectively, for 51 days suffered a rather dry rot; organism recovered.

F. oxysporum 3395; reisolation of strain 2413 from badly rotted Green Mountain potato tuber. Culture used, 4-day-old potato cylinders. Owing to the fact that certain of the tubers were rotting badly, the experiment was concluded before some of the others had started to decay. All of the Pearls, 95 per cent of the Netted Gems, and 50 per cent of the

[1] Orton (9, p. 11) described a soft-rot caused by *Rhizopus nigricans*. Potatoes affected with this disease are called "leaky" or "melters."

Burbanks were rotting after incubation for 25 days at 23.5° C. Four reisolations were made.

In Table I are given the results of inoculations with *F. oxysporum.*

TABLE I.—*Results of the inoculation of different varieties of potatoes with original and reisolated strains of Fusarium oxysporum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
2997	Jersey Peachblow	4	19	23. 1	100
	Idaho Rural	18	19	23. 1	100
	Early Rose	5	19	23. 1	100
	People's	5	19	23. 1	100
2999	Jersey Peachblow	4	19	23. 1	100
	Idaho Rural	17	19	23. 1	100
	Early Rose	6	19	23. 1	100
	People's	6	19	23. 1	100
3045>2997	Netted Gem	9	21	25. 6	100
	Idaho Rural	21	21	25. 6	100
	People's	7	21	25. 6	100
3045>2997	Idaho Rural	4	37	20. 4	100
	Netted Gem	4	37	20. 4	100
	Burbank	4	37	20. 4	100
	Pearl	4	37	20. 4	100
1948	Green Mountain	1	51	17. 8	0
	....do	1	51	18. 4	100
	....do	10	24	24. 6	100
2413	....do	1	51	17. 8	100
	....do	1	51	18. 4	100
	....do	10	14	25. 7	100
3395>2413	Burbank	10	25	23. 5	50
	Netted Gem	19	25	23. 5	95
	Pearl	17	25	23. 5	100

>=reisolation of.

FUSARIUM Wollenw.—*F. hyperoxysporum* 3273; isolated in October, 1914, from a soft-rotting Irish potato from Ocean Springs, Miss. (Pl. XV, figs. 1, 2.) Cultures used for inoculation, pionnotes on 56-day-old culture on stem of *Melilotus alba* and a 10-day-old potato cylinder. After 14 days' incubation at an average temperature of 25.7° C. all tubers inoculated with this species were more or less wet-rotted about the inoculation pricks and the lenticels, two tubers being completely softened. The organism was recovered by four reisolations. Fifty-one days' incubation at temperatures ranging from 16.3° to 18.4° gave a slight rot in all. A gradual increase was observed with the increase in temperature. At 18.4° all were rotted, one being completely destroyed. Four reisolations were made.

F. hyperoxysporum 3343; reisolation of strain 3273, from rotting Green Mountain potato tubers 15 days after inoculation at 25.7°. Culture used, a 26-day-old stem of *Melilotus alba* with pionnotes. All of the inoculated tubers of the four varieties Idaho Rural, Netted Gem, Burbank, and Pearl were rotted after an incubation period of 28 and 37

days at average temperatures of 19.7° and 20.4°, respectively. Seven reisolations were identified.

F. hyperoxysporum 3399; isolated from *Ipomoea batatas* from Lincoln, Ark., by Mr. L. L. Harter. Determined by Miss Ethel C. Field and the author. Culture used for inoculation, 20-day-old cotton stem. As given in Table II, after 51 days' incubation at an average temperature of 21.5°, the results were as follows: Of the four inoculated tubers of each of the varieties Idaho Rural, Netted Gem, Burbank, and Pearl 0, 1, 1, and 4 tubers were rotting, respectively. The organism was recovered by four isolations.

F. hyperoxysporum 3489; reisolation of strain 3399. Culture used for inoculation, 8-day-old potato cylinder and rice culture. This strain was considerably more active than the parent strain 3399. All tubers were rotted after an incubation of 25 days at 23.5°. Six reisolations were made.

Table II gives the results of the inoculations with *F. hyperoxysporum*.

TABLE II.—*Results of the inoculation of different varieties of potatoes with original and reisolated strains of Fusarium hyperoxysporum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
3273	Green Mountain	4	51	16.3	100
		4	51	17.0	100
		4	51	17.8	100
		4	51	18.4	100
		10	14	25.7	100
3343>3273	Idaho Rural	4	28	19.7	100
	Netted Gem	4	28	19.7	100
	Burbank	4	37	20.4	100
	Pearl	4	37	20.4	100
3399	Idaho Rural	4	51	21.5	0
	Netted Gem	4	51	21.5	25
	Burbank	4	51	21.5	25
	Pearl	4	51	21.5	100
3489>3399	Burbank	9	25	23.5	100
	Netted Gem	25	25	23.5	100
	Pearl	22	25	23.5	100

>=reisolation of.

FUSARIUM VASINFECTUM Atk.—Inoculations were made with *F. vasinfectum* isolated from cotton and a similar organism from okra to determine whether this species, which is closely related to *F. hyperoxysporum*, would cause a decay of potatoes. Although considerable decomposition occurred in the inoculated tubers, a scrutiny of the data summarized below reveals the nonconclusive nature of the results obtained.

F. vasinfectum 1855; reisolated by Dr. Wollenweber, in December, 1912, from the vascular system of a cotton plant wilting as a result of

inoculation with strain 1733, a reisolation of strain 1635, which in turn was a reisolation of an original strain 1485 obtained from the discolored vascular system of the main root of a wilting cotton plant from Florence, S. C., on June 15, 1912. Culture used, 26-day-old pionnotes on stem of *Melilotus alba.*

F. vasinfectum 3167; reisolation of 1855, on June 19, 1914, from Idaho Rural potato in above experiment, after 25 days' incubation at 25.5° C. Culture used, 19-day-old pionnotes on a potato cylinder.

The results with tubers inoculated with *F. vasinfectum* 1855 after an incubation period of 25 days at an average temperature of 25.5° were as follows: The five tubers of the Netted Gem variety remained sound; one of the three tubers of the Idaho Rural variety and all of the People's variety were rotted, the organism being recovered from both varieties. With strain 3167, one of these reisolations, only 75 per cent of the tubers of the Pearl variety were rotted after 51 days' incubation at an average temperature of 21.5° C. These tubers were attacked only where a comparatively large cut surface had been exposed to the inoculum. The organism was recovered in each attempt, three reisolations being made.

F. vasinfectum 3263; isolated in September, 1914, as a particularly virulent strain of the cotton-wilt fungus from supposedly wilt-resistant cotton obtained in breeding experiments from Denmark, S. C. Culture used, 20-day-old potato cylinder.

F. vasinfectum 3243; isolated on September 5, 1914, from the vascular bundles of a wilting okra plant from Wrightsboro, N. C. Culture used, 20-day-old potato cylinder.

With *F. vasinfectum*, strains 3263 and 3243, the results were less conclusive. In tubers inoculated with the former strain the organism persisted for 41 days at average temperatures of 18.3° and 18.9° without perceptible damage. Of 10 tubers at 23.5° for 41 days, 5 were rotted, the organism being recovered from 3 of them and *F. radicicola* being isolated from 2. The organism persisted in the other 5 tubers, though no rot resulted. With strain 3243 the organism persisted for 51 days at 17.8° and 18.4° without damage to the tubers. One tuber at 24.6° for 24 days was badly rotted, and the organism was recovered; of 9 tubers at 23.5° for 41 days, only one rotted. The organism was not recovered, but *F. radicicola* was isolated.

In this connection it may be noted that in one experiment (p. 202), which included *F. vasinfectum* 1855 and two strains of *Verticillium albo-atrum* among other organisms, some of the tubers inoculated with the species of Verticillium and likewise certain controls rotted; from these the organism used could not be recovered, but *F. vasinfectum* was isolated several times.

Table III gives the data of the inoculations with *F. vasinfectum.*

TABLE III.—*Results of the inoculations of different varieties of potatoes with original and reisolated strains of Fusarium vasinfectum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
1855	Netted Gem	5	25	25.5	0
	Idaho Rural	3	25	25.5	33
	People's	5	25	25.5	100
3167>1855	Idaho Rural	4	51	21.5	0
	Netted Gem	4	51	21.5	0
	Burbank	4	51	21.5	0
	Pearl	4	51	21.5	75
3263	Green Mountain	1	41	18.3	0
	do	1	41	18.9	0
	do	10	41	23.5	50
3243	do	1	51	17.8	0
	do	1	51	18.4	0
	do	9	41	23.5	10
	do	1	24	24.6	100

>=reisolation of.

JELLY-END ROT AND A TUBER DRY ROT CAUSED BY FUSARIUM RADICICOLA

JELLY-END ROT

"Jelly-end" is the very appropriate name applied by growers to potatoes affected with a field rot and a storage rot which annually cause serious losses in the delta lands of California and in the irrigated sections of Oregon and Idaho.

Many of the tubers when dug show the characteristic soft rot at the stem end, the affected portion easily separating from the rest of the tuber (Pl. XVI, XVII). The rot proceeds uniformly until the whole tuber becomes a slimy mass within the entire skin. If allowed to dry out, the skin sometimes persists as a loose attachment at the stem end, or it may shrivel and wrinkle down on the affected part, in this stage suggesting dry rot.

The jelly-end rot is not a new disease, but nothing has been done to establish the cause of the trouble. Orton (9, p. 5), discussing the wilt and dry end-rot of potatoes in California, says: "An early form of this Fusarium dry end-rot is frequently met with shortly after digging, and potatoes thus affected are known to buyers as 'jelly-ends.'" Shear (13, p. 6) says: "A serious feature of this disease [wilt] is that it forms a means of entrance for other fungous and bacterial diseases of the tubers, such as 'jelly-end' and dry rot." The examination of specimens from different localities indicates that jelly-end rots may be caused by several species of Fusarium. Wollenweber (21, p. 257–258, 264–265) isolated both *F. orthoceras* and *F. radicicola*, and of this disease he says in part (p. 265):

> In Watsonville, Cal., in October, 1913, the writer found up to 80 per cent of Burbank potatoes in a large acreage affected by this peculiar soft rot, which is quite different

from that produced by *F. coeruleum* and other species * * *. In tubers with the jelly-end rot *F. orthoceras* is often, but not always, associated with such fungi as *F. radicicola, Mycosphaerella solani, Sporotrichum flavissimum* Lk., Rhizoctonia, and also with bacteria.

Concerning *F. radicicola*, he says (p. 258):

It is often isolated from Irish potato, especially from dry tubers affected with stem-end dry rot. Sometimes it is associated with other organisms, but frequently seems to invade the tuber from the stolon before a cork layer has been formed * * *. Its presence in the sweet potato suggests that it might require a higher optimum temperature than its related species, such as *F. solani* and *F. martii*.

F. radicicola, *F. oxysporum*, *F. moniliforme* Sheldon, and *Rhizoctonia* sp., together with various saprophytic fungi and bacteria, were isolated by the writer from jelly-end rots from Watsonville and Moorland, Cal. *F. orthoceras*, *Mycosphaerella solani*, and *Sporotrichum flavissimum* were not obtained from such tubers.

F. radicicola was most frequently obtained from typical "jelly-end" tubers from California and Idaho. Its ubiquitous nature and its behavior in all of the inoculation experiments support the view that it is one of the most important causes of this disease. The relation of this species to other tuber rots is discussed in the paragraph on dry-rot.

The prevalence in California of wilt caused by species of Fusarium and the frequency with which *F. oxysporum* was isolated from jelly-end rot suggests the fundamental relationship of this species to the disease. *F. oxysporum* was isolated and identified 24 times from jelly-end rot and stem-end dry-rot tubers from California alone. While often associated with bacteria and fungus saprophytes, in most of these cases it was the only organism secured from the respective tubers which could be regarded as the cause of the condition. It was freqently present in pure culture at the border of rotting and healthy tissues. Whether unaided it produces jelly-end rot under field conditions is not known. A potato tuber from California was diagnosed as ring disease and placed in the incubator. After a period of two months at an average temperature of 18.36° C. a typical jelly-end rot had developed. *F. oxysporum* was the only organism secured from the interior of this tuber at the border of healthy tissue. The inoculation experiments with *F. oxysporum* support the view that it is capable of producing jelly-end rot. *F. radicicola* and *F. oxysporum* were also isolated, though not necessarily in association, from rot areas on the side of tubers resulting from wounds and lenticel invasion.

DRY-ROT

F. radicicola as a cause of stem-end dry-rot was first obtained in August, 1913, from some tubers submitted from Grassfield, Va. Its widespread occurrence in stem-end dry-rotting tubers may be judged from the following distribution: Hermiston, Oreg.; Watsonville and Sonora, Cal.; Fallon, Nev.; Ocean Springs, Miss.; Jerome, Idaho; Honeoye

Falls, N. Y.; Potomac Flats, Washington, D. C.; Arlington, Va.; etc. It enters the stem end of the tubers most commonly, but also invades lenticels and wounds. In some cases the affected tissue is light colored and soft, suggesting bacterial rot—i. e., practically the jelly-end rot. More often in the East it is characterized externally by a firm sunken area with the underlying parenchyma brown to black, dry, tough, and sharply differentiated from the healthy tissue.

This stem-end wound and lenticel dry-rot caused by *F. radicicola* may be regarded as a form of jelly-end rot. The organism is one of the causes of jelly-end rot, but the field and storage conditions where it occurs are different. Under conditions of high humidity the rot is of the jelly-end type; where the humidity or temperature is low and the action of the fungus less rapid, dry-rot develops, the affected tissue being more firm and darker colored as a result of drying and oxidation. (See p. 197, Pl. XV, fig. 4, 5.) Both types occur in California, Oregon, and Idaho, sections under irrigation. The dry-rot phase was the one most frequently submitted for diagnosis from other localities—i. e., of presumably slower development at lower temperatures.

INOCULATION OF POTATO TUBERS WITH FUSARIUM RADICICOLA

F. radicicola 2842; isolated in October, 1913, from jelly-end rot of Burbank potato from Middle River, Cal. Unfortunately, the number of tubers in the experiment with this strain was not recorded. About 1 peck of potatoes of the Burbank variety and ½ peck of the Netted Gem variety were used for inoculation and controls. The tubers were incubated at temperatures ranging from 14° to 20.3°; average lowest compartment, 16.7°; highest, 18.2° C. After 37 days' incubation only one tuber showed a rot; this was at an average temperature of 18.2° C. The organism was recovered.

The thirty-eighth day after inoculation the remaining tubers were exposed to an average temperature of 22.8° C. for the succeeding 19 days. At this time all inoculated tubers were rotted, all stages of wet-rot and dry-rot being represented. The Netted Gems were more badly affected than the Burbanks. In every case the organism was recovered where the attempt was made, four reisolations being identified.

F. radicicola 2890; isolated in October, 1913, from a jelly-end rotted tuber of the Burbank variety from Watsonville, Cal. (associated with *Rhizoctonia* sp. 2892). Culture used, 9-day-old pionnotes on a stem of *Melilotus alba*. All inoculated tubers showed a progressive rot beginning at the inoculation prick (Pl. XVII) after 20 days' incubation at an average temperature of 23° C. The lenticels were invaded and the sprouts infected and dropping off. Some of the tubers were completely softened, only a slimy mass remaining in the entire skin. The organism was recovered by six reisolations.

F. radicicola 2890 plus *Rhizoctonia* sp. 2892. The two organisms were used in combination, 14 tubers being inoculated and incubated as above. More advanced decomposition seemed to take place than when *F. radicicola* alone was present. However, the species of Rhizoctonia could not be recovered, but *F. radicicola* was reisolated wherever the attempt was made.

F. radicicola 3021; reisolation of 2890 from a Burbank potato 20 days after inoculation with the latter. With this reisolated strain an attempt was made to ascertain the effect of the temperature factor on the action of the organism. The inoculated tubers (Irish Cobbler variety) were badly decomposed at average temperatures of 23.3°, 20.2°, and 19.5° C. At 18.7° the majority were more seriously affected than at lower temperatures; indeed, at 17.5° and 15.1° the effect was a slow dry-rot, while at 12.5° the organism persisted for 88 days without perceptible damage to the host.

F. radicicola 3023. Another reisolation of strain 2890; from lenticel infection after 20 days' incubation at 23° C. All tubers of the three varieties Netted Gem, Idaho Rural, and People's inoculated with this strain and incubated for 21 days at an average temperature of 25.6° were very badly decomposed. The organism was recovered by three isolations.

F. radicicola 2998; isolated March, 1914, from a stem-end ring disease and wound-infected tuber from Fallon, Nev. Culture used, 12-day-old pionnotes on stem of *Melilotus alba.* All tubers inoculated with this strain and incubated 20 days at 23° C. rotted. The organism was recovered.

F. radicicola 3236; isolated in August, 1914, in association with *F. hyperoxysporum* from a soft-rotting tuber from Ocean Springs, Miss. Culture used, 1-month-old potato cylinder. The results with this strain are as follows: One tuber incubated for 14 days at 25.7° was badly softened with wet-rot. The organism was recovered. Nine tubers at 24.6° for 24 days were slightly rotted in every inoculation prick, one tuber being completely softened with grayish wet-rot. Organism recovered by two reisolations. Sixteen tubers incubated 51 days at temperatures ranging from 16.3 to 18.4° C. gave the following results: At lowest temperature no rot occurred, but the organism had become established; two of the four tubers at 17° were rotting slightly, with the organism established in the others; at 17.8°, two were slightly rotted, with the organism persisting in the others; at 18.4° one tuber was sound and the three others were rotting.

F. radicicola 2862; isolated October, 1913, from jelly-end rot of a tuber of the Burbank variety from Sargent Island near Middle River, Cal. Culture used, 9-day-old pionnotes on stem of *Melilotus alba.* This strain was comparatively inactive, only 12 per cent of the inoculated tubers rotting after 20 days' incubation at 23° C. The organism was recovered.

F. radicicola 3319; isolated November, 1913, in association with *Mucor* sp. 3320 from a "leaky" diseased potato tuber from Moorland, Cal. Culture used, 1-month-old pionnotes on a potato cylinder. This strain was similar to 2862, being comparatively inactive. After 51 days' incubation at 21.5 C., only 1 tuber of 16 inoculated developed a rot. No attempt was made to recover the organism.

The results of the inoculations with *F. radicicola* are given in Table IV.

TABLE IV.—*Results of inoculation of different varieties of potato with original and reisolated strains of Fusarium radicicola*

Species and strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°*C.*	
Fusarium radicicola 2890.	Burbank.....	20	20	23.0	100
Fusarium radicicola 2890 and *Rhizoctonia* sp. 2892.	.....do.....	14	20	23.0	100
Fusarium radicicola 3201>2890.	Irish Cobbler.....	5	24	23.3	100
		10	88	20.2	100
		10	88	19.6	100
		25	88	18.7	100
		10	88	17.5	100
		6	88	15.1	100
		6	88	12.5	0
Fusarium radicicola 3023>2890..	Netted Gem.....	10	21	25.6	100
	Idaho Rural.....	14	21	25.6	100
	People's.....	4	21	25.6	100
Fusarium radicicola 2998.	Burbank.....	8	20	23.0	100
Fusarium radicicola 3236.	Green Mountain....	4	51	16.3	0
		4	51	17	50
		4	51	17.8	50
		4	51	18.4	75
		1	14	25.7	100
		9	24	24.6	100
Fusarium radicicola 2862.	Burbank.....	25	20	23.0	12
Fusarium radicicola 3319.....	Idaho Rural.....	4	51	21.5	0
	Netted Gem.....	4	51	21.5	25
	Burbank.....	4	51	21.5	0
	Pearl.....	4	51	21.5	0

>=reisolation of.

A NEW DRY-ROT CAUSED BY FUSARIUM EUMARTII

A type of field and storage rot hitherto undescribed was frequently observed in the examination of potatoes from Pennsylvania during the last two years. The character of this rot is as follows: In mild infection of the stem end the tuber shows externally a darkened sunken area with a greenish luster about the stolon insertion. If a thin slice is cut at this point, the parenchyma and the vascular ring are seen to be browned to varying depths. Some of the bundles are discolored to a greater depth

than the parenchyma and are darker in color, sometimes almost black. In this stage the condition might be mistaken, and probably has been in the past, for stem-end ring disease caused by *F. oxysporum* or *Verticillium albo-atrum*, or for one phase of net necrosis (10, p. 14), which it more closely resembles. By the culture method, however, a species of Fusarium is invariably obtained from such tubers at the border of diseased and healthy tissues. The name "*Fusarium eumartii*" is proposed for this fungus.

In the more advanced stages of rot caused by *F. eumartii* the end of the tuber or the entire tuber is involved (Pl. XVIII). According to the humidity and other environmental conditions, the rot is (1) soft and light-brown or (2) dry, corky to friable, and dark-brown to almost black. In general, the rot proceeds uniformly as a sharply differentiated layer easily removable when moist, but close-clinging when dry. In field material the bundles are often discolored as above noted, in advance of the rot. Attempts to isolate the organism from the tips of such bundles usually failed. In the experiments the rot is preceded by a moist water-soaked zone of enzymic activity, from the border of which no organism was obtained. No difficulty was experienced in isolating *F. eumartii* from the border of the discolored tissue and the watery zone.

Considerable care is necessary to differentiate this rot from the one caused by the closely related *F. radicicola*. Sometimes the determination is to be decided only by the careful preparation and study of high cultures. The morphological differences between *F. eumartii* and *F. radicicola* are discussed on page 205.

F. eumartii is chiefly a stem-end and wound invader, but under favorable conditions the lenticels become infected. The fact that *F. oxysporum* was sometimes obtained in association with this fungus and the further fact that this disease of the tubers is reported on plants described as having symptoms of wilt suggest the probable relationship of *F. oxysporum* to the trouble. A field study of wilt and the relation of *F. oxysporum* to such field rots and storage rots should throw considerable light on the problem.

Attempts to isolate an organism from a type of stem-end necrosis similar to mild cases of invasion with *F. eumartii* often failed. There seems to be a sterile necrosis of the stem end, accompanied by browning of the parenchyma and bundles, which is related to the disease described as net necrosis (10, p. 14, pl. 2). Sometimes this type of stem-end necrosis can be distinguished from slight infection with *F. eumartii* only by the culture method; but when the minute ramifications of the vascular ducts are discolored, resulting in the characteristic phase of net necrosis, it can not be confused with the new type of rot.

This rot was obtained chiefly in Pennsylvania, the following localities representing its known distribution: Tower City and Orwigsburg, Schuylkill County, Pa.; East Greenville, Montgomery County, Euclid,

Butler County, and in Dutchess County, N. Y. To judge from correspondence with growers it is a field rot and a storage rot of considerable importance. Infected tubers placed in storage rot badly the following spring; some of the growers are reported to have lost 50 per cent from dry-rot. Whether unaided *F. eumartii* produces a wilt and a rot as a result of planting infected seed is not known. More likely it is secondary to infection by *F. oxysporum* or *Verticillium albo-atrum* in such cases.

INOCULATION OF POTATO TUBERS WITH FUSARIUM EUMARTII

F. eumartii 2932; isolated on January 3, 1914, from a stem-end dry-rotting tuber (Heath's Medium-Late Surprise variety) from Tower City, Pa. Culture used, 7-day old pionnotes on cotton stem.

F. eumartii 2947; isolated as above on January 15, 1914. Culture used, 7-day-old pionnotes on potato cylinder.

F. eumartii 3040; reisolation of 2947, April 23, 1914, from rotting Idaho Rural potato, 19 days after inoculation at 23.1° C. Cultures used, 22-day-old pionnotes on potato cylinder, and in a subsequent trial 2-months-old cultures on rice, *Melilotus alba*, and cotton stems.

F. eumartii 2958; isolated on January 28, 1914, as recorded in Nos. 2932 and 2947. Culture used, 7-day-old pionnotes on potato cylinder.

All tubers of the five varieties mentioned which were inoculated with the several original and reisolated strains of this species of Fusarium showed a progressive rot beginning at the points of inoculation in each case; many of the lenticels were invaded, sunken, and with the subjacent parenchyma browned. People's variety was the most susceptible, the others being affected in the order named—Early Rose, Jersey Peachblow, Netted Gem, and Idaho Rural (Pl. XIX). However, even in the last-mentioned variety there was 100 per cent of infection about the inoculation pricks and lenticel invasion of all tubers. Some of the inoculated tubers were completely softened; others showed a dark-brown zone about the inoculation prick, surrounded by an extensive watery zone of softened tissue. At low temperatures a typical slow dry-rot was produced. The respective organisms were recovered in every attempt made: Nos. 2932 and 2947 from all varieties used; 2958 from the Idaho Rurals; 3040 in first trial, one reisolation from the Idaho Rurals, and one from the Netted Gems; in a later experiment five reisolations were made from the Idaho Rural variety.

Table V gives the results of the inoculations with *F. eumartii*.

TABLE V.—*Results of the inoculation of different varieties of potatoes with original and reisolated strains of Fusarium eumartii*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
2932	Jersey Peachblow	3	19	23. 1	100
	Idaho Rural	14	19	23. 1	100
	Early Rose	4	19	23. 1	100
	People's	4	19	23. 1	100
2947	Jersey Peachblow	4	19	23. 1	100
	Idaho Rural	19	19	23. 1	100
	Early Rose	4	19	23. 1	100
	People's	4	19	23. 1	100
3040>2947	Netted Gem	9	21	25. 6	100
	Idaho Rural	14	21	25. 6	100
	People's	5	21	25. 6	100
3040	Idaho Rural	15	65	13. 8	100
		15	65	17. 2	100
		15	65	18. 6	100
2958	Jersey Peachblow	3	19	23. 1	100
	Idaho Rural	18	19	23. 1	100
	Early Rose	3	19	23. 1	100
	People's	3	19	23. 1	100

>=reisolation of.

CONTROL INOCULATIONS OF POTATO TUBERS

In order to ascertain whether any organism at random would cause a decay of potato tubers under the conditions used to establish the wound-parasitic property of the species mentioned, certain species of Fusarium and other organisms inhabiting potato tubers were included in the experiments. The following organisms were used for this purpose: *F. martii*, *F. solani*, *F. moniliforme*, *Verticillium albo-atrum*, *Sporotrichum flavissimum*, a species of Mucor, and a species of *Rhizoctonia*. The notes on the effect of these organisms on different varieties of potatoes at sundry temperatures are extracted from the several experiments and grouped according to organism as a support of the method. It may be mentioned in this connection that certain strains of *F. radicicola* (Nos. 2862 and 3319) were found to be comparatively inactive under conditions identical with those in which other strains were most virulent.

INOCULATION OF POTATO TUBERS WITH CERTAIN SPECIES OF FUSARIUM AND OTHER TUBER-INHABITING ORGANISMS

F. solani 176; isolated in 1908 by Dr. Wollenweber at Dahlem, near Berlin, Germany, from a potato tuber. Used for the original description of this species by Appel and Wollenweber (1, p. 77). Culture used, 2-months-old pionnotes on potato cylinder. After 51 days at an average temperature of 21.5° C., this organism had attacked only 50 per cent of but one variety, Pearl, and then only where a large cut surface was

exposed. In other words, but 2 tubers of 16 inoculated were rotted. From the two affected tubers *F. solani* was recovered once, *F. radicicola* was isolated twice, and *F. oxysporum* once.

F. martii 186; isolated from *Pisum sativum* in April, 1910. Sent to Dahlem, Germany, by Miss J. Westerdijk, of Amsterdam, Netherlands, as *F. vasinfectum*, var. *pisi* Van Hall; determined by Dr. Wollenweber. Culture used, 2-months-old pionnotes on potato cylinder. None of the 16 tubers inoculated was affected after 51 days' incubation at an average temperature of 21.5° C.

F. moniliforme 3321; isolated on November 3, 1914, in association with *F. radicicola* 3319 and *Mucor* sp. 3320 from a "leaky" (see footnote, p. 190) tuber from Moorland, Cal. Culture used, 1½-months-old cotton stem culture. Of the 16 tubers inoculated, none was rotted after 41 days at an average temperature of 21.5° C.

Verticillium albo-atrum 1717 and 2784. The former strain was isolated by Dr. Wollenweber in September, 1912, from the discolored vascular bundles of wilting okra plant from Monetta, S. C. Strain 2784 was isolated on August 28, 1913, from a wilting potato plant of the Rural variety from Greeley, Colo. After an incubation period of 25 days at an average temperature of 25.5° C. the tubers of the Netted Gem and Idaho Rural varieties inoculated with the respective strains remained sound. The tubers of the People's variety inoculated with these strains were badly rotted in both cases. The organisms could not be recovered, but *F. vasinfectum* was isolated. Tubers inoculated with the latter species were in the same compartment.

Sporotrichum flavissimum 1455; isolated and determined by Dr. Wollenweber in May, 1912; from a hollow Irish Cobbler potato from Arlington, Va. Culture used for inoculation, 2-weeks-old potato cylinder. Of 12 tubers inoculated with the organism and incubated for 20 days at 23° C., none was rotted.

Mucor sp. 3320; isolated on November 3, 1914; from same source and in association with *F. moniliforme* 3321 and *F. radicicola* 3319. Culture used, 2-months-old fruiting culture on cotton stem. Two tubers out of 16 inoculated with this organism were rotted after incubating for 51 days at 21.5° C. From these the organism was recovered by one reisolation, and *F. oxysporum* and *F. vasinfectum* were isolated each once. Tubers inoculated with the latter species were in the same compartment.

Rhizoctonia sp.; for inoculation results with this organism see p. 197.

In Table VI are given the results of the inoculations with the species of Fusarium and other potato-inhabiting organisms.

TABLE VI.—*Results of the inoculation of different varieties of potato tubers with certain species of Fusarium and other tuber-inhabiting organisms*

Species and strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
Fusarium solani 176.	Idaho Rural	4	51	21.5	0
	Netted Gem	4	51	21.5	0
	Burbank	4	51	21.5	0
	Pearl	4	51	21.5	[a] 50
Fusarium martii 186.	Idaho Rural	4	51	21.5	0
	Netted Gem	4	51	21.5	0
	Burbank	4	51	21.5	0
	Pearl	4	51	21.5	0
Fusarium moniliforme 3321.	Idaho Rural	4	41	21.5	0
	Netted Gem	4	41	21.5	0
	Burbank	4	41	21.5	0
	Pearl	4	41	21.5	0
Verticillium albo-atrum 1717.	Netted Gem	4	25	25.5	0
	Idaho Rural	4	25	25.5	0
	People's	5	25	25.5	[a] 100
Verticillium albo-atrum 2784.	Netted Gem	4	25	25.5	0
	Idaho Rural	3	25	25.5	0
	People's	5	25	25.5	[a] 60
Sporotrichum flavissimum 1455.	Burbank	12	20	23.0	0
Mucor sp. 3320	Idaho Rural	4	51	21.5	0
	Netted Gem	4	51	21.5	[a] 25
	Burbank	4	51	21.5	0
	Pearl	4	51	21.5	[a] 25

[a] The respective organism is doubtfully the cause, as in each case wound-parasitic species of Fusarium were isolated in association. See text.

TAXONOMIC ARRANGEMENT AND DIAGNOSTIC CHARACTERS OF IMPORTANT ROT-PRODUCING SPECIES OF FUSARIUM

FUSARIUM Link

The sections Martiella, Elegans, and Discolor provisionally established by Wollenweber (19, p. 32; 20, p. 28) include the species of Fusarium causing tuber-rot known to be economically important. Certain other species—namely, *F. ventricosum*, *F. gibbosum*, *F. culmorum*, *F. orthoceras*, and *F. subulatum*—reported by Wollenweber (19, 20) as weak wound parasites of the Irish potato are not included in the following arrangement of species. *F. solani*, the type species of the section Martiella, is listed because of its ubiquitous occurrence on potatoes as well as on roots and tubers of other plants. Subnormal conidia of *F. coeruleum*, *F. radicicola*, and *F. eumartii* are easily confused with those of *F. solani*. The form, size, and septation of normal conidia must be depended upon for differentiation.

A. SECTION MARTIELLA

[Species in this section are *F. solani* (Mart.) Sacc., *F. martii* App. and Wollenw., *F. eumartii*, n. sp., *F. coeruleum* (Lib.) Sacc., and *F. radicicola* Wollenw.]

1. Fusarium solani (Mart.) Sacc. (1, p. 77).

Conidia normally triseptate (Pl. XIV, fig. 3) up to 100 per cent, occurring in pionnotes and sporodochia,[1] averaging 30 to 40 by 5 to 6μ. Limits of normal triseptate conidia: 25 to 45 by 4.5 to 6.5μ. Seldom 2 and 4, exceptionally 1 and 5 septate (limits: 1-septate, 15 by 4μ minimum; 5-septate, 59 by 6.5μ maximum; greatest width, 7μ; highest septation, 7.) Conidial mass brownish white, becoming brown in age; often greenish as a result of infiltration with greenish blue pigment from the plectenchymatic mycelium. Chlamydospores terminal, intercalary, and conidial; unicellular, round or pear-shaped, 8.5 by 8 μ; 2-celled with constriction at cross wall, 12 by 7.75μ; smooth, rarely in chains or clumps.

Habitat.—On decaying tubers and roots of plants and in the soil. Isolated from species of Solanum, Citrullus, Cucumis, Cucurbita, Lycopersicon, Pinus, Hibiscus, Avena, Zea, Triticum, Panax, Citrus, Pelargonium. Collected by various investigators and identified by Wollenweber and Carpenter.

F. solani (*sensu strict.*) is regarded as a saprophyte, but apparently it acts as a weak wound parasite under exceptionally favorable conditions.

2. Fusarium coeruleum (Lib.) Sacc. (1, p. 90).

Conidia normally triseptate (Pl. XIV, fig. 5), averaging 30 to 40 by 4.5 to 5.5μ (limits of normal triseptate conidia: 23 to 47 by 4.25 to 6μ); seldom 4 and 5 septate (limits: triseptate, 23 by 4.25μ minimum; 7-septate, 58 by 5.75μ maximum). Conidial mass brownish white and yellow ocher to reddish ocher. Plectenchymatic stroma chiefly violet to indigo blue and bluish black; by infiltration with the latter color the conidial masses may become bluish green, as in other species of the section Martiella. *F. coeruleum* is the only species of the section having reddish ocher conidial masses. Chlamydospores as in other species of the section.

Habitat.—On tubers of *Solanum tuberosum*. Established as a cause of tuber rot in this country and in Europe by Wollenweber (20, p. 44). Determined by Dr. Wollenweber and the writer in material from the following localities: Ottawa, Canada; Houlton, Me.; Rhinebeck, N. Y.; Fredericksburg, Md.; Norfolk, Va.; Parkersburg, W. Va.; Donnybrook, N. Dak.; Idaho Falls, Idaho; Potlatch, Wash.; and several places in Oregon.

3. Fusarium eumartii, n. sp.

F. eumartii isolated from the Pennsylvania dry-rot agrees with Appel and Wollenweber's (1, p. 78–84) diagnosis of *F. martii* except in certain details of the conidia. The latter in the new species are higher septate and have a somewhat larger average size (Pl. XIV, fig. 4). Normally 4 to 6 septate, averaging 54 to 75 by 5.5 to 6.6μ (limits: 50 to 80 by 5 to 7.2μ). Largest conidia 85 by 7.2μ (7 and 8 septate). Percentages of variously septate conidia, average sizes and limits as found in a 10-day-old pionnotes on *Melilotus alba* and in a 15-day-old pionnotes on cotton are given in Table VII.

[1] For definition of these terms see Wollenweber, H. W. (20, p. 24).

TABLE VII.—*Percentages of variously septate normal conidia, average sizes, and limits of size as found in a 10-day-old pionnotes on Melilotus alba and in a 15-day-old cotton pionnotes of Fusarium eumartii.*

10-DAY-OLD PIONNOTES ON MELILOTUS ALBA

Septation.	Percentage of conidia.	Average size of conidia.	Limits.
		μ	μ
3	7	.....	
4	20	54.4 by 5.6	51 to 54.4 by 5.1 to 6.1.
5	50	63.7 by 5.8	59.5 to 69.7 by 5.4 to 6.1.
6	8	69.7 by 6.3	66.3 to 71.4 by 6.1 to 6.8.
7	15	71.6 by 6.5	68 to 76.5 by 5.9 to 6.8.

15-DAY-OLD PIONNOTES ON COTTON

3	5	.....	
4	17	.....	
5	58	62.9 by 6.1	56 to 76.5 by 5 to 6.8.
6	18	73.2 by 6.6	51 to 81.6 by 5.9 to 7.2.
7	2	79.9 by 6.6	74.8 to 85 by 6.3 to 6.8.
8	Rare.	85 by 6.8	

The formation of pigment in *F. eumartii* (Pl. A, fig. 6–8) and *F. radicicola* is much the same as that in *F. solani*, only more gorgeous. The conidial color fluctuates between brownish white and bright brown; by infiltration of the greenish blue plectenchymatic pigment the conidial mass becomes gray, blue-green, to brown and a dark mixed color. The plectenchymatic stroma is weakly developed or lacking, and therefore the pionnotes lies naked on the substratum. The chlamydospores, 7 to 10μ in diameter, agree with those in other species of this section.

F. eumartii causes a rot of potatoes in experiments, while *F. martii* is said to be a saprophyte (20, p. 30). This statement was confirmed with *F. martii* 186 collected in Germany. The new species agrees more closely with *Fusisporium solani* Martius (8) in the size of conidia than does *F. martii*.

F. radicicola and *F. eumartii* are very closely related to *F. martii* with respect to average size and septation of normal conidia and occupy the same relative positions on either side of the last-mentioned species as a type. In average measurements the conidia of *F. radicicola* are approximately 30 per cent shorter and 20 per cent narrower than those of *F. martii* (*sensu strict.*), while *F. eumartii* is larger in about the same proportion. *F. radicicola* is typically triseptate, *F. martii* 3- to 4-septate, and the new species 5- to 6-septate. Similar constant varieties of certain other species are known—e. g., of *Fusarium solani*.

Habitat.—On decaying tubers of *Solanum tuberosum* from Pennsylvania and New York. Cause of potato dry-rot and wet-rot.

4. **Fusarium radicicola** Wollenw. (21, p. 257–258).

The conidia of this species are normally triseptate, averaging 30 to 45 by 3.75 to 5μ; narrower than in *F. solani, sensu strict.* (Pl. XIV, fig. 3), and shorter and fewer septate than in *F. martii* and *F. eumartii* (Pl. XIV, fig. 4). The plectenchymatic mycelium, as in the two latter species, is olive colored on potato cylinders, shading to green and brown. Pionnotes on potato cylinders, cotton, and stems of *Melilotus alba* brownish white to blue and verdigris (Pl. A, fig. 6–8). Pigment formation the same as in *F. martii* and *F. eumartii*. Chlamydospores as in other species of the section.

Habitat.—On partly decayed tubers and roots of plants. Cause of potato dry-rot and jelly-end rot. Identified from the following: *Ipomoea batatas* (collected by Mr. L. L. Harter); *Musa sapientum* (collected by Mr. S. F. Ashby, Jamaica, Porto Rico); *Cucumis sativus* (collected by Mr. F. V. Rand, West Haven, Conn.); soil (collected by Mr. F. C. Werkenthin, Austin, Tex.).

B. SECTION ELEGANS

[Species in this section are *F. oxysporum* Schlecht., *F. hyperoxysporum* Wollenw., *F. vasinfectum* Atk., *F. tracheiphilum* Sm., *F. niveum* Sm., *F. lycopersici* Sacc., *F. conglutinans* Wollenw., *F. redolens* Wollenw., *F. orthoceras* App. and Wollenw., *F. orthoceras*, var. *triseptatum* Wollenw., *F. batatatis* Wollenw.]

1. **Fusarium oxysporum** Schlecht. (20, p. 28).

2. **Fusarium hyperoxysporum** Wollenw. (21, p. 268).

F. oxysporum (Pl. XIV, fig. 1) is not sharply differentiated morphologically from several species of this section—namely, *F. hyperoxysporum*, *F. vasinfectum*, *F. tracheiphilum*, *F. lycopersici*, and *F. niveum*. *F. hyperoxysporum* forms a perfect pionnotes in contrast to the reduced pionnotes in *F. oxysporum* (Pl. A, fig. 1–5). According to Harter and Field (4, p. 296), it is different biologically in that it causes a stem-rot of *Ipomoea batatas* and is not infectious on young plants of *Solanum tuberosum*, while *F. oxysporum* causes a wilt of the latter host but does not attack the former (21, p. 268). Both develop a lilac odor on starchy media. However, this character is of doubtful specific value since non-odor-forming strains of *F. oxysporum*, *F. hyperoxysporum*, and *F. vasinfectum* have been isolated, and some of the odor-forming strains temporarily lose this property in culture.

F. tracheiphilum, the cause of a wilt of species of Vigna, is without pionnotes and odor. *F. vasinfectum*, the cause of a wilt of cotton, develops a perfect pionnotes of an ocherous-salmon color; on potato cylinders in subdued light this color becomes slightly purple. Typically a strong lilac odor is present on starchy media. A non-odor-forming strain was designated *F. vasinfectum*, var. *inodoratum*, by Wollenweber (20, p. 29). *F. lycopersici*, the cause of a wilt of *Solanum lycopersicum*, differs from *F. oxysporum* in having conidia of a little larger average size and produces colorless sclerotial plectenchymatic masses in contrast to the bluish masses of this sort in *F. oxysporum*, etc. No odor is developed. *F. niveum*, to which the wilt of species of Citrullus is attributed, differs from *F. lycopersici* in forming blue sclerotial bodies on potato cylinders; from *F. oxysporum* in having larger conidia and no odor.

It is possible to determine the six above-mentioned species by morphological characters alone. Although a knowledge of the host of the particular species to be determined is not necessary, such information greatly facilitates the work. In spite of the fact that each of these forms seems to cause a wilt on one particular host, it should be pointed out that too much dependence on the value of the host in descriptions of species has led to the present confusion in the nomenclature of the form genus Fusarium.

A species of Fusarium causing a field soft-rot of Irish potatoes in Mississippi (Pl. XV, fig. 1, 2) was morphologically identical with *F. oxysporum* (Pl. XIV, fig. 1), but developed a perfect pionnotes on potato cylinders (Pl. A, fig. 4); thus, it must be identical with *F. hyperoxysporum*, the cause of stem-rot of the sweet potato. Inoculation with *F. hyperoxysporum* isolated by Harter and Field from the latter host resulted in complete destruction of the tubers (see No. 3399 and reisolation of same, No. 3489, p. 192), indicating the truth of the hypothesis.

Further cross-inoculation work carefully controlled by morphological studies should demonstrate whether all of the above-mentioned species of this section are biologically distinct; whether, for example, *F. hyperoxysporum* differs sufficiently from *F. oxysporum*, on the one hand, and *F. vasinfectum*, on the other, to be entitled to the rank of species.

C. SECTION DISCOLOR

[Species in this section are *F. discolor* App. and Wollenw.; *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw.; *F. culmorum* (W. G. Sm.) Sacc. (syn., *F. rubiginosum* App. and Wollenw.); *F. trichothecioides* Wollenw.; and *F. incarnatum* (Rob.) Sacc.]

1. **Fusarium discolor, var. sulphureum** (Schlecht.) App. and Wollenw. (1, p. 115–118).

F. discolor, var. *sulphureum*, is morphologically the same as *F. discolor* App. and Wollenw. (1, p. 114). Normal conidia (Pl. XIV, fig. 6) 3- to 5-septate, 23 to 39 by 4.5 to 5.5μ (limits: 16 to 48 by 3.5 to 6μ); exceptionally 1- and 2-septate. True chlamydospores are rare. Conidial masses ocherous to ocherous-orange. Differs from *F. discolor* in the color of the plectenchymatic mycelium, which never becomes carmine-red (Pl. B.), but changes from ocherous to yellow (egg-yellow to sulphur-yellow, which color permeates the aerial mycelium and conidial masses).

Habitat.—In hollows of potato tubers. Established by Dr. Wollenweber as the cause of a tuber-rot in Germany. It was isolated from decaying tubers from Newell, S. Dak., and identified by Dr. Wollenweber. The writer also identified it in similar material from Cresbard, S. Dak., and in tubers from the North Dakota Agricultural College (collected by Mr. D. G. Milbrath).

2. **Fusarium trichothecioides** Wollenw. (5, p. 146–152).

F. trichothecioides, in contrast to the other species of the section Discolor, forms two sorts of conidia: (1) The comma type, formed as a slightly curved comma ellipsoidally rounded on both sides; and (2) the normal macroconidia, typical of the section. The plectenchymatic mycelium and conidial masses are rosy white, in contrast to the carmine [1] mycelium in *F. discolor* (Pl. B, fig. 1–3) and the ocherous-yellow mycelium in *F. discolor*, var. *sulphureum* (Pl. B, fig. 4–6). The conidial masses in both the last-named species are ocherous orange.

Habitat.—Dry-rotting tubers of *Solanum tuberosum*, causing decay, especially under storage conditions. Geographic distribution: Spokane, Wash.; St. Paul, Minn.; Dayton, Iowa; Alliance, Nebr.; Spearfish, S. Dak. (Jamieson and Wollenweber). The following localities are added to the above: Jerome and Idaho Falls, Idaho; Newell, S. Dak.; and Sioux City, Iowa.

SUMMARY

(1) A new stem-end and wound-invading dry-rot of the Irish potato annually causing serious damage in Pennsylvania is caused by a species of Fusarium for which the name "*Fusarium eumartii*" is proposed.

(2) Another widely prevalent dry-rot similar to the above is caused by *F. radicicola* Wollenw.

(3) *F. radicicola* and *F. oxysporum* are most commonly associated with the so-called "jelly-end" rot, annually a serious trouble in the tule lands of California. The former seems to be the cause in most cases, but the fundamental relationship of *F. oxysporum* to this and other tuber-rots should not be overlooked.

(4) Experimental inoculations show that *F. oxysporum* and *F. hyperoxysporum*, species of the section Elegans, which has been reported as containing purely vascular parasites, are capable of entirely destroying potato tubers.

(5) *F. oxysporum* is the cause of certain types of tuber-rot.

[1] Jamieson and Wollenweber (5) give "purple" mycelium through error.

(6) *F. radicicola* caused no rot at 12° C. A constant storage temperature below 50° F. would prevent the action of *F. radicicola*, *F. eumartii*, and *F. oxysporum*.

(7) The following species of Fusarium are added to those known to cause tuber-rot through wound infection: *F. radicicola* Wollenw.; *F. eumartii*, n. sp.; *F. oxysporum* Schlecht.; and *F. hyperoxysporum* Wollenw.

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PLATE A

Fusarium spp. on vegetable media:

Fig. 1–3 and 5.—*Fusarium oxysporum* Schlecht. 3045. 1, Twenty-one-day-old culture on potato cylinder showing typical bluish green sclerotial masses, no pionnotes. 2, Eighteen-day-old culture on stem of *Melilotus alba* with pionnotes. 3, Eighteen-day-old rice culture with typical coloration of the section Elegans. 5, Thirty-day-old cotton-stem culture with sporodochia.

Fig. 4.—*F. hyperoxysporum* Wollenw. 3343. Thirty-one-day-old culture on potato cylinder with development of pionnotes. Cultures on the three other media are as illustrated for *F. oxysporum* (fig. 1–3, 5).

Fig. 6–8.—*F. radicicola* Wollenw.; illustrates equally well *F. martii* and *F. eumartii*. 6, Potato cylinder 34 days old with pionnotes brown to verdigris. 7, Seventeen-day-old culture on stem of *Melilotus alba* with pionnotes and immature sporodochia. 8, Rice 28 days old, with pionnotes on upper surface. Coloration of the section Martiella.

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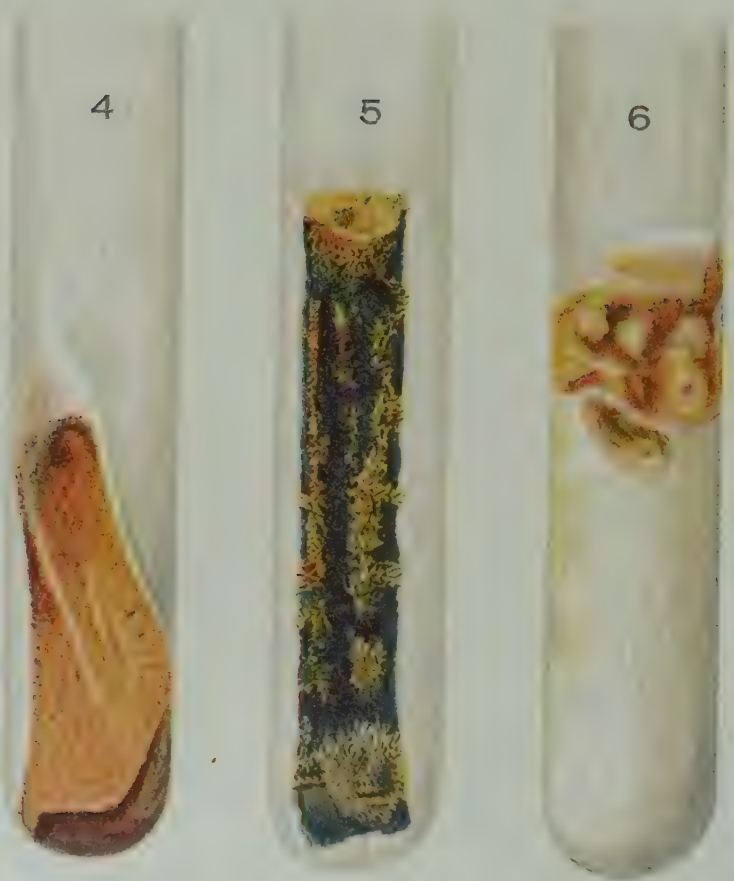
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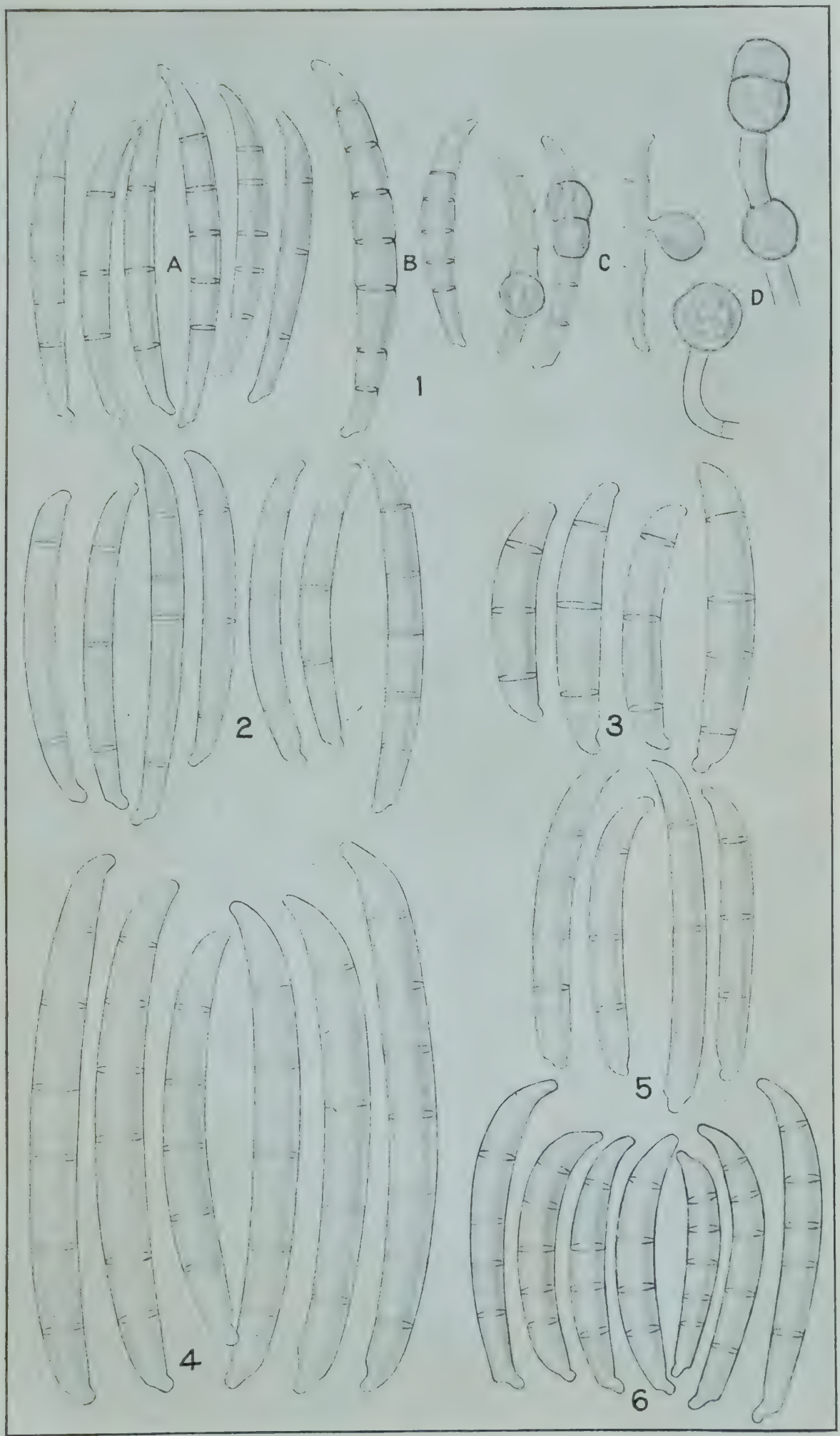
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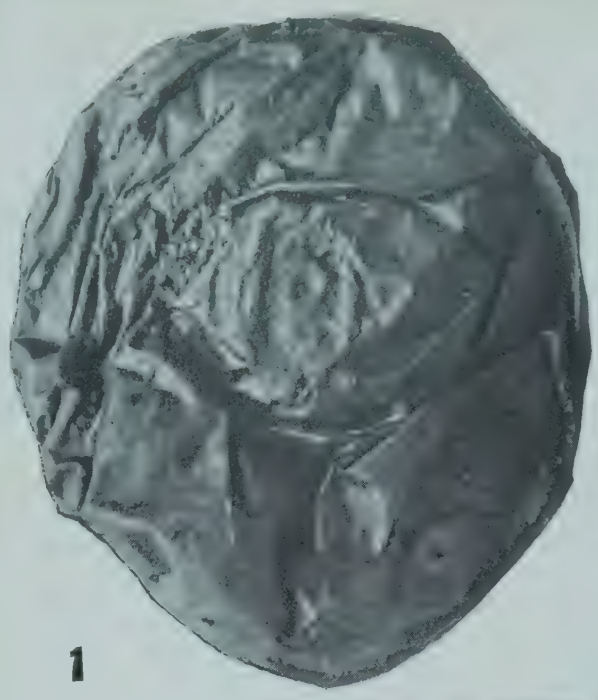

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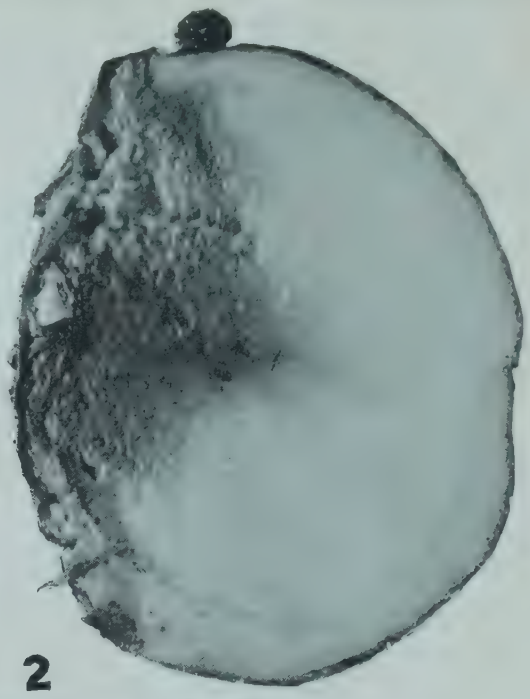

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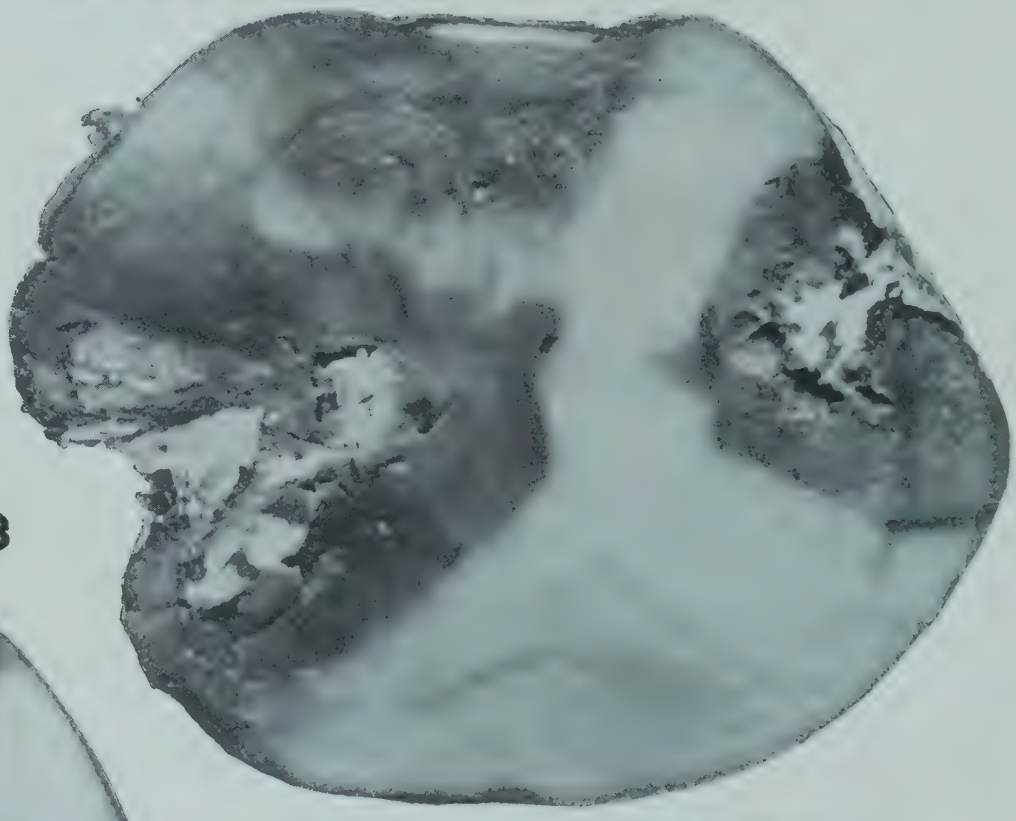

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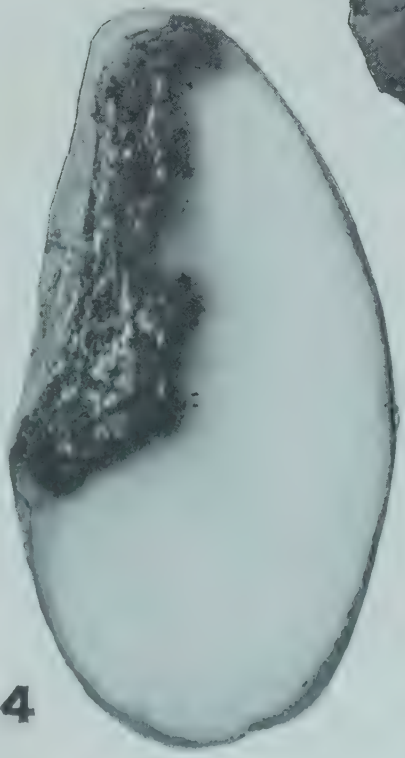

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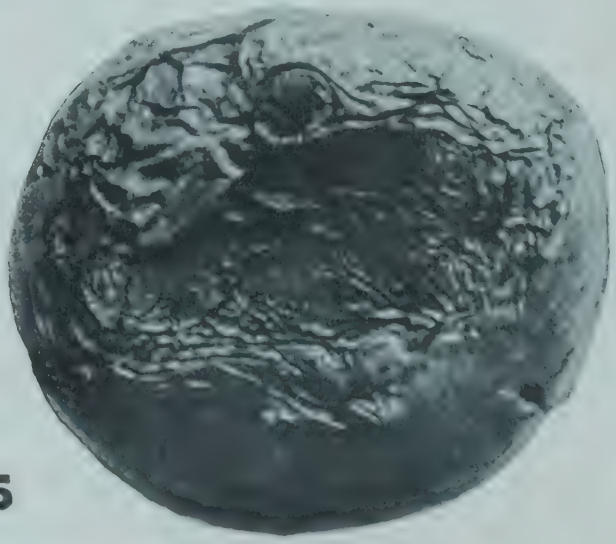

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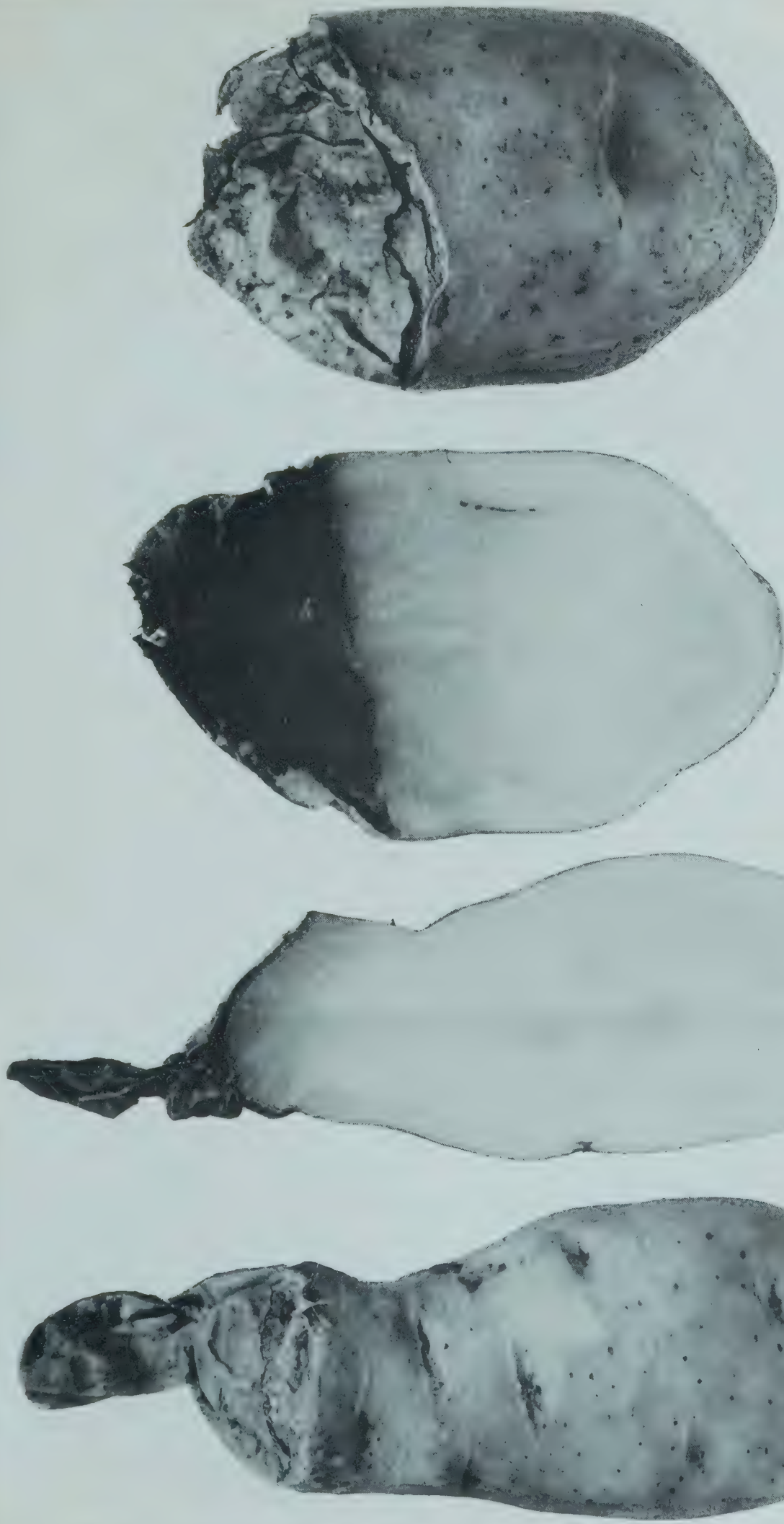

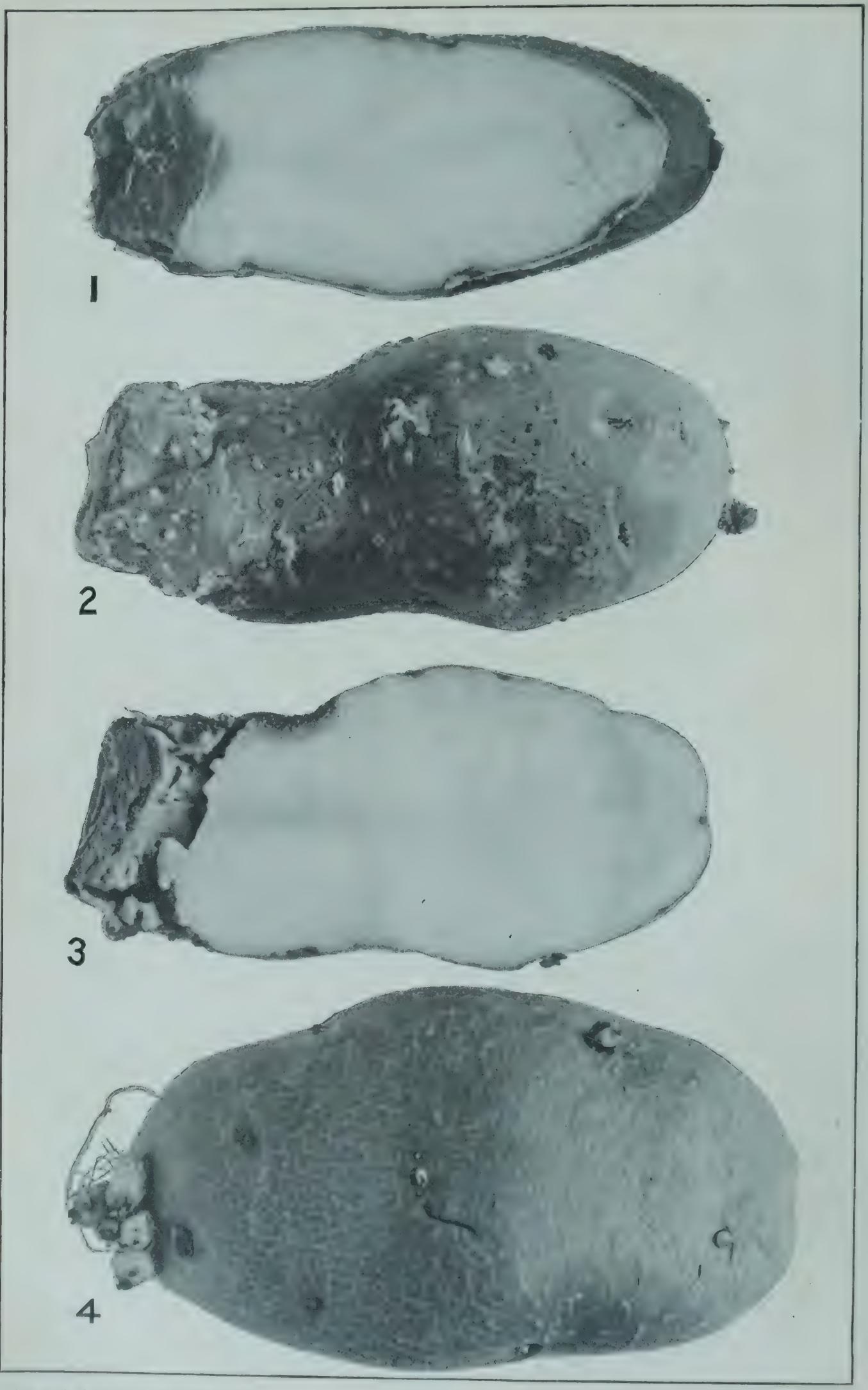
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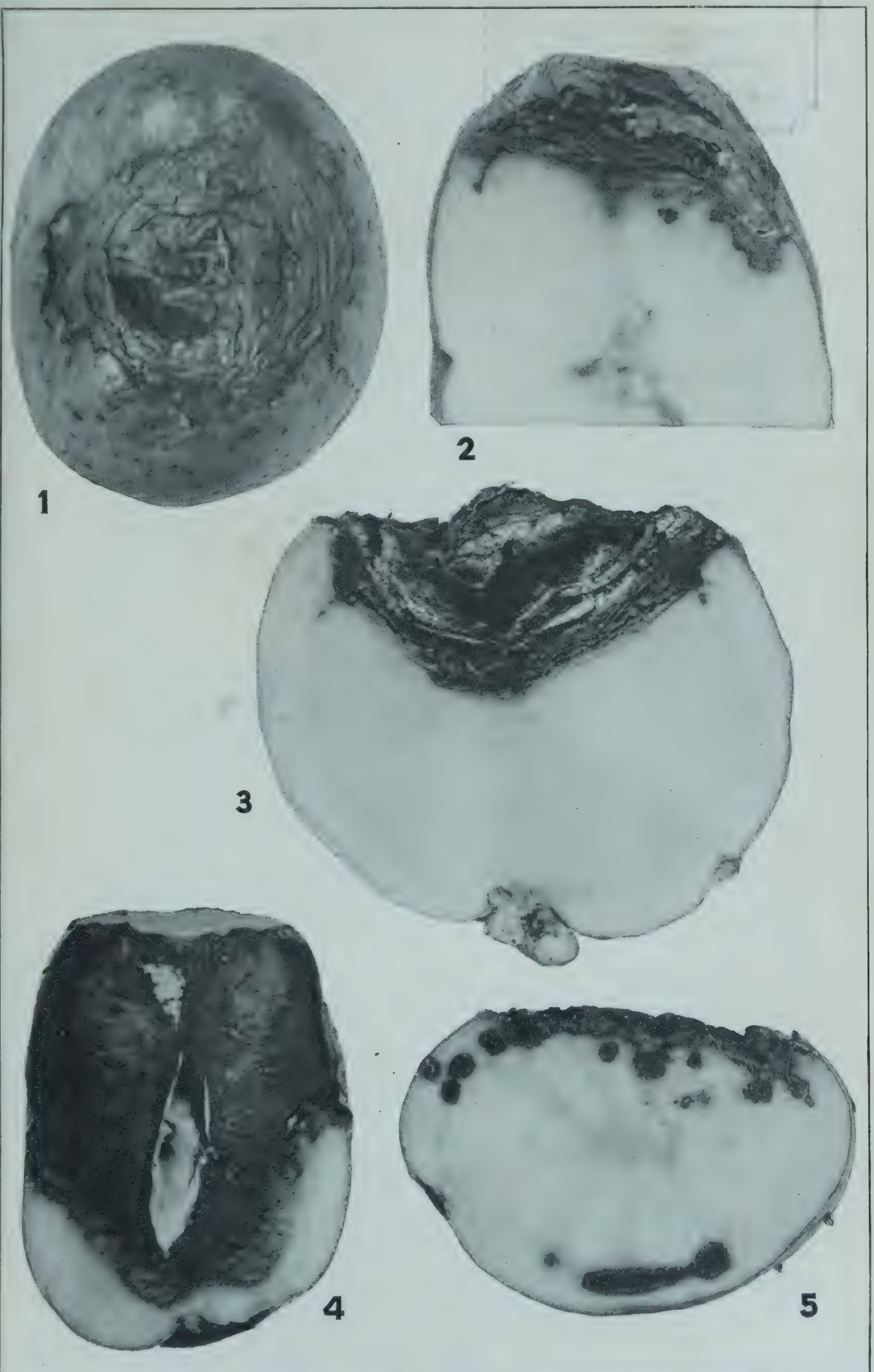
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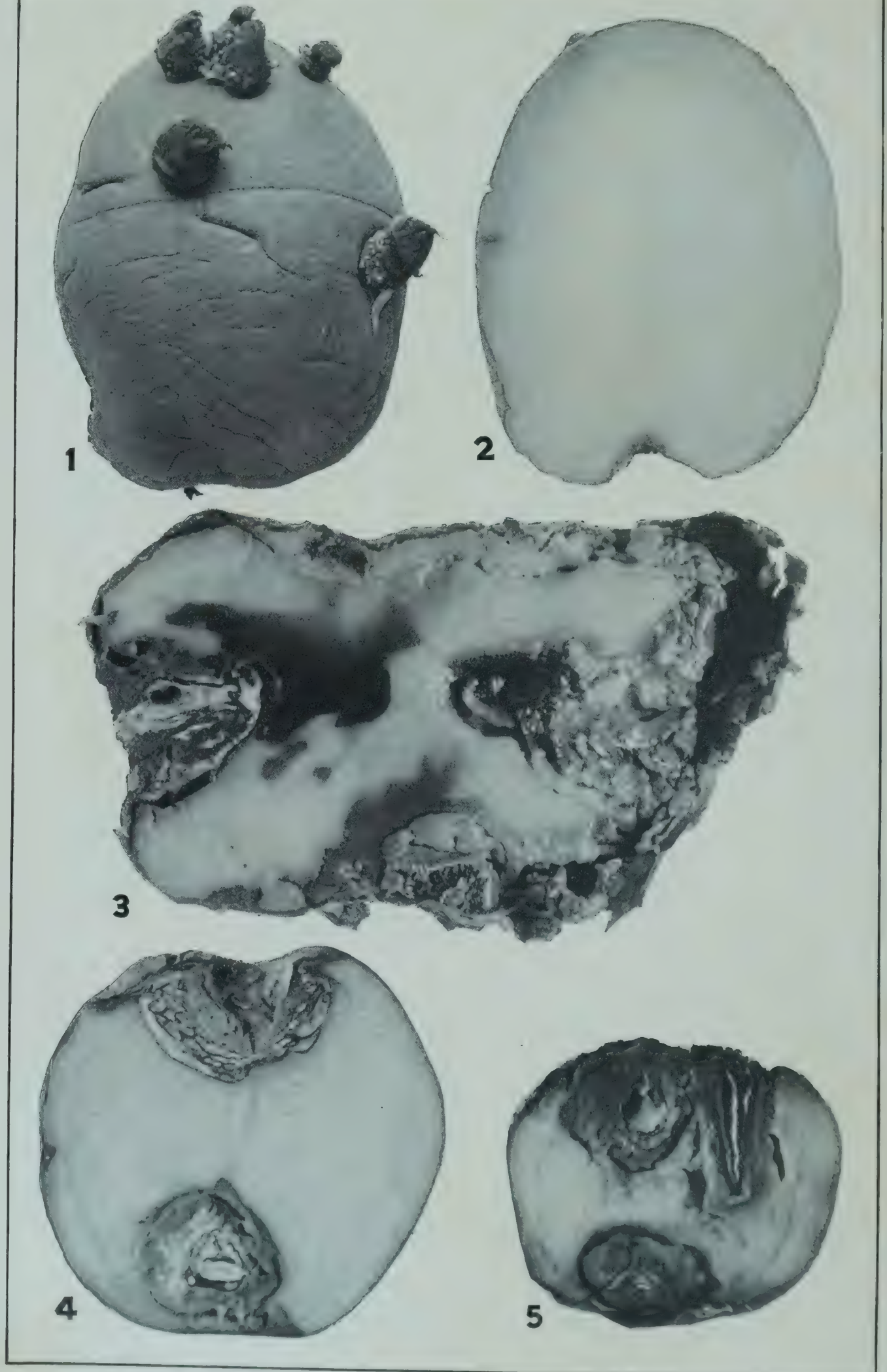
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INFECTION EXPERIMENTS WITH TIMOTHY RUST

By E. C. Stakman, *Head of the Section of Plant Pathology and Bacteriology*, and Louise Jensen, *Mycologist, Division of Botany and Plant Pathology, Department of Agriculture, University of Minnesota*

INTRODUCTION

There is some diversity of opinion as to whether or not timothy rust should be regarded as a distinct species. Eriksson and Henning (2, p. 140–142)[1] in 1894 designated it "*Puccinia phleipratensis* Eriks. u. Henn." Johnson (4) decided that timothy rust in this country was the same as that in Sweden and favors giving the fungus specific rank. Kern (5, 6), on the other hand, thinks it should be considered as a physiological species, or, at most, a variety or subspecies.

It is therefore of interest to know the infection capabilities of the rust. Eriksson and Henning (3, p. 136–141), reported the successful infection of rye (*Secale cereale*) and oats (*Avena sativa*), but none of wheat (*Triticum vulgare*) or barley (*Hordeum vulgare*). Johnson (4, p. 9) obtained results confirming those of Eriksson and Henning. Johnson also succeeded in successfully infecting a number of grasses. He found that the rust would not transfer directly to barley, but if transferred first to oats and then to barley infection resulted. In the same way *Dactylis glomerata* acted as a bridging form between timothy and wheat. Mercer (7) was unable to obtain successful infection on wheat, rye, and various grasses as a result of inoculations made with timothy-rust urediniospores.

The inoculations made by the writers were all on seedlings. The leaves were first thoroughly moistened either with an atomizer or by rubbing water on with the fingers. The spores were applied with a flat inoculating needle. The plants were then placed in shallow pans of water and kept covered with bell jars for 48 hours. The grass seeds were obtained from the Minnesota Seed Laboratory. The following varieties of cereals were used: Oats, Improved Ligowa, Minn. No. 281; barley, Manchuria, Minn. No. 105; wheat, Bluestem, Minn. No. 169; rye, Swedish, Minn. No. 2.

RESULTS OF INOCULATIONS

The writers made a number of inoculations with timothy-rust urediniospores, the results of which are given in Table I.

[1] Reference is made by number to "Literature cited," p. 216.

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TABLE I.—*Results of inoculations with timothy-rust urediniospores on cereals and grasses*

Date of inoculation.	Source of urediniospores.	Plant inoculated.	Number of leaves inoculated.	Number of leaves infected.
Dec. 18, 1914	*Phleum pratense*	*Triticum vulgare*	37	0
Dec. 24, 1914	do	do	20	0
Dec. 29, 1914	do	do	41	0
Jan. 26, 1915	do	do	52	0
Dec. 18, 1914	do	*Avena sativa*	25	3
Dec. 24, 1914	do	do	20	0
Jan. 9, 1915	do	do	23	14
Jan. 17, 1915	do	do	107	19
Feb. 19, 1915	do	do	20	1
Dec. 18, 1914	do	*Hordeum vulgare*	31	0
Dec. 24, 1914	do	do	21	2
Dec. 29, 1914	do	do	31	11
Jan. 26, 1915	do	do	48	3
Feb. 1, 1915	do	do	23	8
Dec. 18, 1914	do	*Secale cereale*	11	0
Dec. 24, 1914	do	do	14	0
Dec. 29, 1914	do	do	38	1
Feb. 1, 1915	do	do	39	1
Feb. 19, 1915	do	do	41	6
Apr. 3, 1915	do	*Avena fatua*	7	2
Apr. 11, 1915	do	do	10	2
Apr. 3, 1915	do	*Avena elatior*	20	3
May 29, 1915	do	*Bromus tectorum*	23	2
Jan. 12, 1915	do	*Dactylis glomerata*	55	37
May 14, 1915	do	*Elymus virginicus*	40	1
Apr. 11, 1915	do	*Lolium italicum*	21	0
May 29, 1915	do	do	32	3
Do	do	*Lolium perenne*	15	4
Do	do	do	40	4
Mar. 3, 1915	*Hordeum vulgare* [a]	*Phleum pratense*	48	0
Mar. 14, 1915	do	do	40	0
Mar. 7, 1915	*Avena sativa* [b]	do	71	0
Apr. 11, 1915	*Avena fatua*	do	34	0
Apr. 18, 1915	do	do	15	0
May 2, 1915	*Phalaris canariensis* [c]	do	25	0
Mar. 7, 1915	*Dactylis glomerata*	do	40	0
Mar. 14, 1915	do	do	56	0

SUMMARY.

Source of inoculating material.	Plant inoculated.	Result of inoculation.[d]	Source of inoculating material.	Plant inoculated.	Result of inoculation.[d]
Phleum pratense	*Triticum vulgare*	0/150	*Phleum pratense*	*Lolium italicum*	3/53
Do	*Avena sativa*	37/195	Do	*Lolium perenne*	8/55
Do	*Hordeum vulgare*	24/154	Do	*Bromus tectorum*	2/23
Do	*Secale cereale*	8/143	*Hordeum vulgare*	*Phleum pratense*	0/88
Do	*Avena fatua*	4/17	*Avena sativa*	do	0/86
Do	*Avena elatior*	3/20	*Avena fatua*	do	0/34
Do	*Dactylis glomerata*	37/55	*Phalaris canariensis*	do	0/25
Do	*Elymus virginicus*	1/40	*Dactylis glomerata*	do	0/96

[a] *Puccinia graminis*, originally from *Hordeum jubatum;* on barley 8 urediniospore "generations."
[b] *Puccinia graminis*, originally from *Dactylis glomerata;* on oats 9 urediniospore generations.
[c] *Dactylis glomerata* rust after 13 generations on oats and one generation on *Phalaris canariensis.*
[d] The denominator gives the total number of leaves inoculated, the numerator the number which developed pustules.

It will thus be seen that the rust from timothy transfers directly to three of the common cereals. Neither Eriksson and Henning (3) nor Johnson (4), as previously mentioned, were able to obtain successful infection on barley as a result of direct transfer from timothy. However, the writers were able to infect some plants in four of the five series of inoculations. The percentage of infections on barley is nearly as great as that on oats and is greater than that on rye. The rust transferred very readily to *Dactylis glomerata* and fairly well to both *Avena elatior* (*Arrhenatherum elatius*) and *Avena fatua*. It also transferred to *Lolium perenne*, *Lolium italicum*, and *Bromus tectorum*. One extremely small pustule developed on *Elymus virginicus*.

The vigor of infection varied greatly on different hosts. In addition to the inoculations indicated in Table I, many inoculations were made on timothy. These nearly always resulted in a 100 per cent infection. The incubation period on timothy was 7 to 8 days, while on barley it was 10 to 12 days. It was clearly evident that barley was an uncongenial host; fairly large dead areas were frequently formed without subsequent development of pustules, and all pustules, when they did develop, were extremely small. Most of the pustules were less than 1 mm. in diameter, being mere dots in some cases. However, others were somewhat larger, some attaining a diameter of over 1 mm. On oats the pustules were larger, the rust developing in a fairly normal manner. The pustules on rye were fairly small, but there was not such a distinct tendency to produce flecks as there was on barley. The infection on *Avena elatior*, *Avena fatua*, *Lolium perenne*, and *Lolium italicum* was moderate, while that on *Dactylis glomerata* was very severe, nearly as severe as that on timothy. On *Bromus tectorum* the pustules were extremely small.

Although the rust transferred fairly readily from timothy to both barley and oats, no infection was obtained on timothy as a result of inoculations with *Puccinia graminis hordei* and *Puccinia graminis avenae*. Less than 100 inoculations were made with *Puccinia graminis hordei;* in no case, however, was there any indication of successful infection. The transfer is entirely possible; more inoculations will therefore be made. Timothy was inoculated directly with *Puccinia graminis avenae*, but no infection resulted from any of 86 trials. No better results were obtained by transferring first to *Avena fatua*, *Phalaris canariensis*, or *Dactylis glomerata*. None of these forms, therefore, acted as a bridging form between oats and timothy. It is possible that such bridging forms may exist, although the possibility has not yet been demonstrated. Carleton (1, p. 62) reported successful infection of *Puccinia graminis avenae* on *Phleum asperum*. It is possible that this form might act as a bridging species, but the writers have not yet had opportunity to determine this.

EXPERIMENTS WITH BRIDGING HOSTS

Johnson (4, p. 10) found that by using *Avena sativa* as a bridging host the timothy rust could be transferred to *Hordeum vulgare;* by using *Festuca elatior* it could be transferred to *Hordeum vulgare* and to *Triticum vulgare;* by using *Dactylis glomerata* it could be transferred to *Triticum vulgare.* Since the writers were able to infect barley directly, but not wheat, without the bridging hosts, an attempt was made to determine whether or not, with the strain of rust employed, it would be possible to make transfers to wheat after using *Dactylis glomerata* as a bridging form and whether or not the rust would transfer to barley more readily under the same conditions. Transfers were made from timothy to *Dactylis glomerata,* and heavy infection was obtained. Two series of inoculations were then made with spores from *Dactylis glomerata* to wheat, oats, barley, rye, and timothy. The results were as follows: Wheat, $\frac{0}{19}$; oats, $\frac{2}{38}$; barley, $\frac{8}{36}$; rye, $\frac{2}{32}$; timothy, $\frac{12}{20}$. When oats was used as a bridging host, approximately the same percentage of infections resulted as when the rust was transferred directly from timothy. The writers were, therefore, unable to increase the infection capabilities of the rust by means of first transferring to *Dactylis glomerata* or oats. Neither was the vigor of infection appreciably greater on barley and oats after using bridging species. It is possible that by confining the rust for a long series of generations on a bridging host definite results might be obtained. Such experiments are now under way.

The results cited show that different results may be obtained with different strains of rust. That Johnson (4) and Eriksson and Henning (3) worked with different strains seems entirely probable, in view of the fact that neither was able to transfer the rust directly to barley, while the writers experienced no particular difficulty in making such transfer. The possibility of conflicting results may be clearly shown by results which the writers have recently obtained. Timothy rust and stem rust of oats (*Puccinia graminis avenae*) transferred very readily to *Dactylis glomerata.* But the rusts by no means acquired the same capabilities as a result of growing on *Dactylis glomerata,* at least not in a few generations. When the timothy rust on *Dactylis glomerata* was transferred to oats less than 10 per cent of the inoculated leaves became infected; when the rust was transferred to barley very small pustules were produced on about 16 per cent of the inoculated leaves; when it was transferred to rye, small pustules were produced on about 6 per cent of the inoculated leaves; when it was transferred to timothy 95 per cent of the leaves became infected. When, on the other hand, stem-rust of oats (*P. graminis avenae*) on *Dactylis glomerata* was transferred to oats, 100 per cent of the inoculated leaves became very severely affected; inoculations on barley resulted in 7 per cent of infection; inoculations on rye resulted in no infection (in other experiments the writers have been able to infect

rye with *P. graminis avenae*); no infection resulted from inoculations on timothy. The writers also have two strains of *Puccinia graminis*, both of which have been confined to the same variety of barley for nine months. Both attack barley and a number of wild grasses very readily; neither has ever infected oats; one attacks wheat with extreme vigor and infects rye only with difficulty, while the other is almost entirely unable to infect wheat but attacks rye with great vigor.

It seems fairly clear that, as Johnson (4, p. 10) has previously pointed out, timothy rust and *Puccinia graminis avenae* are quite similar. Both rusts transferred to *Dactylis glomerata*, *Avena fatua*, *Avena elatior*, barley, rye, *Lolium perenne*, *Lolium italicum*, *Bromus tectorum*, and *Elymus* spp.; the oats rust to *Elymus robustus* and *Elymus canadensis;* and the timothy rust to *Elymus virginicus*. With the exception of *Avena fatua*, they transferred with somewhat the same degree of readiness.

MORPHOLOGY OF THE SPORES

Morphologically, however, the two rusts are somewhat different, the spores of *Puccinia graminis avenae* being larger. Spores of *Puccinia graminis avenae*, originally from *Dactylis glomerata* and then confined to oats for 14 successive generations, ranged from 19 to 35μ in length and from 16 to 24μ in width, the modes falling at about 30 and 19μ. The spores of timothy rust on timothy ranged from 17 to 31μ in length and from 14.5 to 23μ in width, the modes falling at about 26 and 18μ. After one generation on *Dactylis glomerata*, the timothy-rust spores ranged from 17 to 32μ in length, and from 13.5 to 23.2μ in width, while the modes fell at about 25.5 and 19.5μ. At least 100 spores from different pustules were measured. Measurements were also made of spores produced after the rust had been one generation on other hosts, including oats, rye, barley, *Lolium perenne*, and *Avena fatua;* but no distinct and consistent differences were apparent, with the exception of the spores produced on barley. These were smaller than those produced on any other host, ranging from 18.5 to 28.3μ in length and from 13 to 20μ in width. The modes were at about 23 and 17μ. Whether or not greater variations would occur if the rust were confined to the different hosts for longer periods of time is not yet known. Experiments have been begun to determine the effect of different hosts on the morphology of the spores.

SUMMARY

(1) Timothy rust was transferred successfully directly from timothy to *Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Avena fatua*, *Avena elatior*, *Dactylis glomerata*, *Elymus virginicus*, *Lolium italicum*, *Lolium perenne*, and *Bromus tectorum*.

(2) Attempts to increase the infection capabilities of the rust by the use of bridging hosts for short periods of time were unsuccessful.

(3) The infection capabilities of timothy rust are quite similar to those of *Puccinia graminis avenae.*

(4) Attempts to infect timothy with *Puccinia graminis avenae* and *Puccinia graminis hordei* were unsuccessful.

(5) The morphology of the spores of timothy rust on different hosts varies slightly; spores produced on barley were considerably smaller than those produced on more congenial hosts.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., NOVEMBER 8, 1915 No. 6

EXPERIMENTS IN THE USE OF CURRENT METERS IN IRRIGATION CANALS

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INTRODUCTION

Comparisons of the relative accuracy of measurements made in irrigation canals with current meters using different methods are made in the following discussion. In connection with field experiments made on the flow in various types of canals in order to determine the value of the coefficient n of Kutter's formula,[1] detail current-meter gagings were necessary. These detail gagings and other observations made at the same time have been used to compare the results obtained by the standard two-point, single-point, and integration methods, as well as by floats and various selected points of measurement. Much experience is now available in regard to the various methods of current-meter observations used in natural channels. The results given here apply to the more regular artificial channels used in irrigation for which there are fewer available data.

In the experiments referred to, the current-meter readings were carefully taken at from 12 to 20 points horizontally across the canal section, from four to six readings being made at each point. These detail or multiple-point observations were plotted, and the mean velocity at the different points observed was determined from the vertical velocity curves drawn through the plotted observations. The points across the canal at which observations were made are referred to in the following discussion as the "verticals." The results secured by the multiple-point reading both in each vertical and for the discharge as a whole have been taken as the correct velocities and discharges in the comparisons made. In canals of the size used in most of these experiments determinations of

[1] Ganguillet, E., and Kutter, W. R. General Formula for the Uniform Flow of Water in Rivers and Other Channels; translated from the German, with . . . additions . . . by Rudolph Hering and J. C. Trautwine. ed. 2, 240 p., pl. New York, 1893.

the discharge by other methods than by the use of the current meter would not have been practicable. Greater detail regarding the methods used and the experiments in general will be found in a recent publication,[1] which discusses the results of the determinations of the value of n in Kutter's formula. The field work was carried on by various members of the Division of Irrigation Investigations, as stated in the bulletin referred to.

COMPARISONS OF DIFFERENT METHODS OF MEASUREMENT OF VELOCITIES IN THE VERTICALS

There are four principal methods by which the velocities in different verticals are determined with the current meter: The multiple-point method; the mean of the velocities at the 0.2- and 0.8-depth points, called the "two-point method"; the velocity at 0.6 depth, called the "single-point method"; and the vertical-integration method.

As the main purpose of these experiments was the determination of the value of n, it was desired to make the discharge determinations with as great accuracy as possible. The multiple-point method was used, readings being taken usually at six points in each vertical. This was assumed to give the correct discharge and is the discharge used as the basis of the following comparisons.

The multiple-point readings were usually taken at 0.1, 0.2, 0.4, 0.6, 0.8, and 0.9 of the depth. The meter was held from 30 to 60 seconds at each point. From these measurements the discharge by the two-point or the single-point method was computed and compared with the results of the multiple-point method.

When the field measurements were made, in most of the experiments gagings were also made by the vertical-integration method. Generally one or two complete round trips were made with the meter at each vertical, the vertical movement being from 3 to 16 feet per minute. Much care was used to give the meter a uniform vertical velocity so that each portion of the section would be equally represented in the integrated mean. Two complete round trips were usually made, consuming from 40 to 150 seconds, depending on the depth. The meter was generally moved more slowly in the shallower sections in order to give a sufficiently long time for the reading.

In Table I are given the general results for all experiments. These are divided in five different classes of canal sections, although there is no marked variation for the different groups. These include nearly 100 experiments for the two-point and the single-point methods on canals having discharges of from 2 to 2,600 second-feet. Only 55 experiments

[1] Scobey, F. C. The flow of water in irrigation channels. U. S. Dept. Agr. Bul. 194, 68 p., 9 fig., 20 pl. 1915. *See also* Scobey, F. C. Behavior of cup current meters under conditions not covered by standard ratings. *In* Jour. Agr. Research, v. 2, no. 2, p. 77–83. 1914.

with integration methods are shown, as measurements by this method were not taken in all cases.

TABLE I.—*Variation in discharge in percentage by the two-point, the single-point, and the integration method, compared with the multiple-point method*

Type of canal cross section.	Two-point method.			Single-point method.			Integration method.		
	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation.	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation. 5 per cent correction applied.	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation.
Rectangular flumes....	27	+0.68	1.45	27	+4.90	2.21	17	+1.06	1.36
Concrete-lined trapezoidal sections.....	15	+ .86	1.42	15	+4.21	1.94	4	+ .72	.93
Shallow earth canals, sloping sides.....	13	− .38	1.08	13	+3.11	3.42	9	− .81	2.44
Shallow earth canals, steep sides.....	25	+1.05	1.74	25	+5.02	2.44	18	+ .36	2.15
Earth canals, relatively deep sections.....	16	+1.07	1.70	15	+6.32	3.18	7	+3.06	3.78
Mean of all.....	96	+ .73	1.51	95	+4.80	2.54	55	+ .76	2.07

Table I shows all three methods to give an average discharge greater than the multiple-point gaging. For the two-point and integration methods this is not large, being about three-fourths of 1 per cent for both of these methods. For the single-point method the average error is +4.80 per cent. This is large enough to warrant a correction factor, so that all further comparisons with this method are based on a correction of −5 per cent made to the discharge secured by the single-point method.

Besides the average error of the series of experiments, the probable or average variation of a single observation is also given. While the mean difference of the two-point and integration from the multiple-point method is the same, the single measurements show a somewhat greater average variation for the integration than for the two-point method. If the results of the single-point observations are reduced by 5 per cent, the corrected results have an average variation but little in excess of the other methods. These results may be expressed by saying that with the two-point method a series of observations will give results three-fourths of 1 per cent too high. If no correction is made to the results, single observations will have an average error of 1.5 per cent.

The experiments covered a wide range of discharges and canal types, so that further classifications were made to determine the effect, if any, of differences in the velocity, the depth, or the value of n on the accuracy of the different methods. The results are given in Table II.

TABLE II.—*Comparisons of variations in percentage of discharge by two-point, single-point, and integration methods from discharge by multiple-point methods for different velocities, depths, and values of n*

COMPARISONS FOR DIFFERENT VELOCITIES

Observation.	Two-point method.			Single-point method (corrected by −5 per cent).			Integration method.		
	Number of experiments.	Mean difference from multiple-point.	Average variation of a single experiment.	Number of experiments.	Mean difference from multiple-point.	Average variation of a single observation.	Number of experiments.	Mean difference from multiple-point.	Average variation of a single observation.
Velocities in feet per second:									
Less than 1.00	5	+1.02	2.56	5	+1.64	3.68	3	+2.60	3.16
1.00 to 1.50	18	+ .63	1.59	18	+ .43	2.90	11	+1.62	1.75
1.50 to 2.00	12	− .38	1.60	13	+ .24	2.67	10	+1.43	2.80
2.00 to 2.50	20	+ .89	1.54	19	− .01	2.66	10	− .02	2.51
2.50 to 3.00	14	+1.67	1.68	13	− .25	1.67	10	− .18	1.41
3.00 to 4.00	15	+1.11	1.41	15	− .14	1.91	9	+ .50	1.81
Over 4.00	12	+ .02	.73	12	−2.66	2.83	2	− .43	.72
Mean	96	+ .73	1.51	95	− .20	2.54	55	+ .76	2.07

COMPARISONS FOR DIFFERENT DEPTHS

Mean depth of canal section in feet:									
Less than 1.00	14	−0.65	2.06	14	+1.02	3.66	10	+1.98	2.65
1.00 to 1.50	18	+ .21	1.23	17	+ .53	1.90	8	+1.46	1.65
1.50 to 2.00	15	+1.32	1.73	15	− .03	2.51	7	+ .86	1.49
2.00 to 2.50	22	+1.29	1.58	22	− .10	2.82	12	+1.08	2.81
2.50 to 3.00	16	+ .97	1.25	16	− .77	1.95	12	− .60	1.84
Over 3.00	11	+1.09	1.19	11	− .79	2.44	6	− .26	1.33
Mean	96	+ .73	1.51	95	− .20	2.54	55	+ .76	2.07

COMPARISONS FOR DIFFERENT VALUES OF *n*

Value of *n* in Kutter's formula:									
Less than 0.013	13	+0.40	0.76	13	−1.70	2.27	5	+0.90	0.90
0.013 to 0.017	18	+ .52	1.42	18	− .32	2.73	11	+ .23	2.25
0.017 to 0.021	20	+ .72	1.45	20	− .85	2.16	13	− .52	1.52
0.021 to 0.025	13	+ .80	2.17	13	+1.81	2.52	6	+1.87	2.91
0.025 to 0.029	11	+ .53	.88	11	− .41	2.56	5	−1.30	1.55
Over 0.029	11	+ .66	1.72	10	− .41	3.00	5	+4.35	4.35
Mean	86	+ .61	1.41	85	− .35	2.50	45	+ .59	2.14

The two-point method appears to give results equally accurate for all velocities, depths, and values of *n*, the variations which occur not being seemingly dependent on any of these three factors. The probable error of a single observation is generally less for the large velocities and

depths, which is also true of the other methods. This is to be expected, as the smaller velocities and depths usually occurred in canals of small discharge, where the general conditions for the use of the current meter are not so favorable. The accuracy does not appear to be affected by the character of the channel or value of n.

There is some indication that the correction to be used with the single-point method should be greater than 5 per cent for low velocities and less for the higher ones. This tendency is not marked, however, and it is doubtful if it is sufficient in amount or that it is sufficiently proved by these results to warrant the use of different corrections; also the correction seems to vary with the depth in a similar way.

The integration method seems to give the closest average results for velocities from 2 to 3 feet. It also appears to be more accurate for the greater depths. This latter result is to be expected. In the use of the integration method the velocity in from 0.2 to 0.3 foot in depth must be either missed entirely or imperfectly determined both at the bottom and at the water surface. The velocity at the bottom is lower than the average. Therefore the measurements in the remaining portions of the depth would give results above the actual average velocity. As the proportion of the depth for which velocities are undetermined is larger in the shallow canals, the proportionate error would be greater.

Another method sometimes used is that known as the three-point method, in which the velocity is measured at 0.2, 0.6, and 0.8 of the depth. This is more usually computed by giving the velocity at 0.6 depth equal weight with the mean of the 0.2 and 0.8 depth velocities. As Table I shows the single-point method to be less accurate than the two-point, there is no apparent advantage in the three-point method over the two-point. In sections where the two-point method gave results too low and the single-point too high, their combination might increase the accuracy over that secured by the two-point method alone. Where both were of the same sign, the use of the three-point method would give less accurate results than the two-point alone. The two-point and single-point methods gave results having opposing signs on less than one-third of the total number of experiments, so that the three-point would seem to have little advantage over the two-point method.

To definitely determine the relative accuracy of the three-point method, the discharge of each experiment was computed, using both the method by which the velocity at 0.6 depth is averaged with the mean of the velocities at the 0.2 and 0.8 depths, and also the method by which the velocities at the three points are given equal weight. This latter method would seem to be the more logical, as it has been shown that the two-point, or 0.2 and 0.8 depth method, gives results more accurate than the 0.6 point alone, so that in the use of the three points it would be preferable to reduce the weight given to the velocity at 0.6 depth.

The results of this comparison are given in Table III, which shows that the second method of computation gives the more accurate results. In no class of canal section does either three-point method give as accurate average results as the 0.2 and 0.8 depth method alone. In the individual experiments in one-seventh of the total number the $\frac{0.2+0.8+2\times0.6}{4}$ method gave more accurate results than the 0.2 and 0.8 depth alone. In one-fifth of the total number the $\frac{0.2+0.8+0.6}{3}$ method gave results more accurate than the 0.2 and 0.8 depth alone. These were for gagings in which the errors of the 0.2 and 0.8 depth method were of different sign from those of the 0.6 method, so that their combination reduced the actual error. These cases were generally for canals of irregular section and flow, and indicate that for unfavorable conditions of current-meter work the three-point method may be preferable to the two-point, but that for usual conditions the two-point alone is preferable. However, under unfavorable conditions of irregular velocity and cross section only detail multiple-point observations can be depended upon for accurate results. The $\frac{0.2+0.8+0.6}{3}$ method is always preferable for computation of the results to the $\frac{0.2+0.8+2\times0.6}{4}$ method.

TABLE III.—*Variation in discharge in percentage by the three-point method compared with the multiple-point method*

Type of canal cross section.	Number of observations.	Average variation from multiple-point method.	
		Giving velocity at 0.6 depth equal weight with mean of velocities at 0.2 and 0.8 depths. Mean velocity = $\frac{0.2+0.8+2\times0.6}{4}$	Giving velocities at 0.2, 0.6 and 0.8 depths, equal weight. Mean velocity = $\frac{0.2+0.6+0.8}{3}$
Rectangular flumes	21	+2.5	+1.8
Concrete-lined trapezoidal sections	15	+2.7	+2.0
Shallow earth canals, sloping sides	11	+1.7	+1.3
Shallow earth canals, steep sides	21	+2.5	+2.0
Earth canals, relatively deep sections	14	+3.5	+2.7
Mean of all	82	+2.6	+2.0

MEASUREMENTS WITH SURFACE FLOATS

In many experiments measurements with surface floats were made in order to secure data from which the proper coefficients for use with such measurements could be derived. It is often convenient to make such approximate measurements by timing floats over a known length of canal and applying some coefficient to the product of the velocity so

secured and the cross section of the canal in order to give the discharge. In such measurements there are two principal sources of error: (1) The cross-sectional area is difficult to obtain except in flumes or lined canals of uniform cross sections and (2) mistakes may be made in choosing a coefficient to be used in reducing the maximum surface velocities as obtained from the floats to the mean for the whole canal.

The following results relate to the proper coefficient to be used to reduce surface-float velocities to the mean velocity for the whole cross section. The average errors discussed are those arising from the determinations of float velocities and the choice of coefficients and do not include errors in determining the canal cross sections. For the other purposes of these experiments the areas of the canal sections were carefully determined. In the usual field use of float methods there may be a considerable error introduced due to errors in the approximate determinations of canal cross sections of variable dimensions, which would give larger probable errors for the discharge than would result from the probable error due to the choice of the coefficient to use with the velocity of the float alone.

Various formulas have been derived for the relation of the surface velocity to the mean velocity. These have been derived both for the relation of the surface velocity to the mean velocity in any single vertical in the section and for the relation of the maximum surface velocity to the mean velocity of the whole channel. Ganguillet and Kutter [1] give a formula, deduced by Bazin, in which the ratio of the maximum to mean velocities in a channel are made to vary with

$$\sqrt{\frac{RS}{V^2}}$$

As this term is equal to the C in Chezy's formula, a table is given for the value of the ratio for different values of C. In this formula Kutter substitutes the values of n and R from his general formula and gives a table for the values of the ratio of mean to maximum velocity, depending on R and n. The formula derived by Bazin, which forms the basis of this table, was based on 61 series of gagings.

In the canal experiments discussed in this paper in which float measurements were made several small floats would be started simultaneously at scattered points in the portion of the channel having the highest velocities. The time of the most rapid float was used to compute the maximum surface velocity. This gives lower coefficients than would be obtained by the use of the average of all floats. Small floats such as twigs or chips were used which would have both a small submergence and a small exposed surface above the water. It was found that there was little difference in the velocities of the floats thrown into the main threads of the canals unless some became caught in noticeable side

[1] Ganguillet, E., and Kutter, W. R. Op. cit.

eddies. The floats were generally timed over the 500 to 1,000 feet of canal used in the value-of-n experiment.

The value of the coefficient for each experiment was compared with the coefficient given in Kutter's table for the same value of R and n. For all measurements the coefficients differed by an average of 0.06. The mean of all observations was 0.013 lower than Kutter's. This is not an unreasonable variation when it is remembered that at best the method is only an approximate one.

The selection of the coefficient based on the value of R and n is not, however, a convenient one for field use. The determination of the canal cross section, except for flumes and lined sections, will be approximate and the determination of the value of R even more uncertain. A variation of the coefficient with the water area would be the most convenient for field use. A field measurement involves the determination of the mean cross section of the canal and the velocity of the float. If the selection of the proper coefficient is based on the cross section and an estimated value of n no additional measurements or computations are required in order to select the proper coefficients. The experiments give evidence that the coefficient varies with the character of the wetted surface, so that some knowledge of the value of n is required.

In order to determine the value of the coefficients for different conditions, the results of each measurement were plotted with the value of the coefficient and the cross-section area as coordinates. A series of curves for the different values of n were fitted to these plotted observations and adjusted until they gave results equaling, on the average, the results of the actual field determinations. From these curves the values of the coefficients given in Table IV were secured. No attempt was made to derive an equation for the variation in the value of the coefficient, graphical methods being used throughout.

TABLE IV.—*Coefficients to be applied to velocities of floats to obtain mean velocity in canals*

Area of water cross section.	Value of n in Kutter's formula.									
	0.012	0.014	0.016	0.018	0.020	0.022	0.024	0.026	0.028	0.030
Square feet.										
2	0.85	0.80	0.76	0.73	0.70	0.67	0.65	0.63	0.61	0.60
4	.86	.81	.77	.74	.71	.68	.66	.64	.62	.61
6	.87	.82	.78	.74	.71	.68	.66	.64	.63	.62
8	.88	.83	.79	.75	.72	.69	.67	.65	.63	.62
10	.88	.83	.79	.76	.73	.70	.68	.65	.64	.63
15	.89	.84	.80	.77	.74	.71	.69	.66	.65	.64
20	.90	.85	.81	.78	.75	.72	.70	.67	.66	.65
25	.91	.86	.82	.78	.75	.73	.71	.68	.66	.65
30	.91	.86	.82	.79	.76	.73	.71	.68	.67	.65
35	.91	.86	.82	.79	.76	.73	.71	.69	.67	.66
40	.91	.86	.82	.79	.76	.73	.71	.69	.67	.66
50	.91	.86	.82	.79	.76	.73	.71	.69	.67	.66
Over 50	.91	.86	.82	.79	.76	.73	.71	.69	.67	.66

From these experiments it appears that the coefficient is constant for different values of n for cross-section areas over about 35 square feet. The rate of variation of the coefficient is greatest for the smaller channels. The observations for cross-sectional areas over 100 square feet were too few in number to give dependable averages for canals of larger size, but both these results and Bazin's formula indicate that the coefficient is practically constant for such larger cross sections.

Similar curves were also obtained based on the value of the coefficient and the discharges. These were similar in form and indicate that the velocity within the limits of the experiments did not materially affect the ratio of maximum surface to mean velocity. These values are not given, as the coefficients based on canal areas are more convenient to use.

The results were further classified by the shape of the channel. Apparently the coefficient does not vary with the form of cross section, as the coefficient from the curves agrees fairly well with the observations when the proper values of n are used, whether the canal is rectangular or irregular or whether the section is deep or shallow relative to its width.

The average variation of the observed coefficients from the curves was 0.045. The average of all observations agreed with the curves, the plus variations equaling those of minus sign. Expressed as a percentage, the average variation was 6. For any single observation the observed value of the velocity coefficient is as likely to differ from the mean curve by less than 0.045 as it is to differ by more than this amount. For the average values of the coefficient this amounts to a variation of 6 per cent. In 17 of the 92 experiments, or 18.5 per cent of the total number, the observed value differed by over 10 per cent from the curves.

The more usual practice where such methods of measurements of velocities by floats are made is to use some general value of the coefficient, usually 0.80 or 0.85. These experiments, as well as the observations given by Kutter, clearly indicate that the coefficient varies quite materially for different-sized canals and for different values of n. These results give values for the coefficients which are less than 0.80 for all values of n over 0.016, becoming as low as 0.60 for small canals having high values of n.

The value of n for any given canal is, of course, uncertain to some extent. The coefficient varies most rapidly with the lower values of n. An error of 0.002 in selecting the value of n makes a difference of 5 per cent in the value of the correct coefficient to be used for low values of n, and less than 2 per cent for the higher values.

The coefficients to be used should be selected from the cross-section area and the value of n. The character of the canals corresponding to the different values of n given in Table IV can be secured from the general list following:

Values of n

0.012. Straight wood flume in good condition; clean concrete lining having very smooth finish; no moss or gravel.

0.014. Ordinary straight wood flumes, little rock or sand; unplastered concrete lining; no moss or gravel.

0.016. Worn wood flumes containing growths or sand and gravel; average concrete linings, irregular finish, moss growths or gravels; best earth canals, uniform silted and clean sections.

0.018. Very poor wood flumes; rough concrete with covering of moss or gravel; very good earth canals; uniform section, silted, free from gravel and moss.

0.020. Concrete in poor condition, much moss and gravel; better than average earth sections without growths and fairly regular sections.

0.022. Earth sections, generally free from moss or gravel.

0.024. Average earth canals, fairly clean and regular, some gravel and vegetation.

0.026. Earth canal; gravel and some cobbles, some moss, irregularities in cross section; masonry-lined canals.

0.028. Canals with some cobbles; moss and other unfavorable conditions.

0.030. Earth canals, much moss or weeds, irregular section, gravel or cobbles; fairly smooth rock cuts.

It is preferable to make float measurements on straight portions of canals. If it is necessary to use a length containing curves, a coefficient should be selected for a value of n about 0.002 higher than would otherwise be used.

These experiments give data both on the most probable coefficients to be used in float measurements and also on the limitations of accuracy to be expected. Such measurements are often desirable for quick approximate determinations. The most rapid of several floats should be used and the proper coefficient selected to fit the conditions. The error from the float determinations should not often exceed 10 per cent, although error in estimating the cross-sectional area may result in much larger errors in the resulting discharge for earth canals. In flumes or section of regular forms the error in determining the water area should not be large.

EFFECT ON ACCURACY OF CURRENT-METER GAGINGS FROM THE USE OF DIFFERENT NUMBERS OF OBSERVATIONS ACROSS THE WIDTH OF CANALS

The number of verticals across a gaging station at which velocity measurements should be made is a question on which there has been much difference of opinion.

In the sections of irrigation canals at which current-meter gagings are generally made, the cross section is more regular than in the usual stream gaging station, so that usually fewer measurements should be required. In the experiments discussed, measurements were made in from 13 to 20 verticals with a minimum distance apart of the verticals of 0.5 foot on the smaller canals. These measurements are more than are usual in general field practice. The results obtained were compared with the

discharge which would have been obtained had a less number of verticals been measured. The different types of canal sections were grouped into general classes. For each gaging, discharges using only every other vertical measured were computed and also using only every fourth vertical. Two computations of each gaging using the two sets of alternate verticals were made, and also two sets for every fourth vertical. These results were then compared with the discharge obtained by the use of all the verticals measured, in order to determine the probable errors to be expected when fewer verticals were used. The average number of verticals observed in the experiments was 16; the number in the comparisons averages 8 and 4. In general field current-meter work, if only 8 or 4 verticals had been measured, the ones used might have been located in the cross section differently from the arbitrary method used in this computation, so that the selection of alternate verticals as used should give errors larger rather than smaller than are to be expected. The results of this comparison are given in Table V.

TABLE V.—*Effect on the accuracy of current-meter gagings of varying numbers of verticals*

Type of canal.	Number of detail gagings made.	Average number of verticals in detail gagings.	Comparisons using one-half of observed verticals. Variation (per cent).		Comparisons using one-fourth of observed verticals. Variation (per cent).	
			Average.	Minimum and maximum.	Average.	Minimum and maximum.
Flumes, vertical sides	23	15	0.9	+0.05 to −3.82	2.9	0 to −7.50
Concrete-lined canals; steeply sloping sides	11	14	.9	−.04 to −2.95	2.9	−1.08 to −5.85
Concrete-lined canals; wide and flatly sloping sides	6	17	1.4	−.37 to −3.22	3.8	−.70 to −6.52
Average earth canals, sloping sides	18	16	2.9	+.1 to −8.3	9.2	−1.5 to −17.6
Average earth canals, steep sides	21	16	2.5	+.1 to −7.3	9.0	−.4 to −21.1
Earth canals, relatively deep sections	10	16	2.7	−.5 to −5.5	7.7	−.6 to −19.4
Mean of all	89	16	1.9	.....	6.2	.....

Table V gives both the average difference in percentage and the range of variations in single gagings. Occasionally the use of a less number of verticals may give a greater discharge than that obtained from a more detailed gaging, owing to irregularities in the cross section or velocity. Where an average of 4 verticals were used, less than 2 per cent of the observations gave larger discharges than the use of all verticals, so that the average difference is practically equal to the mean error. Where 8 verticals were used for all observations, one in each seven measurements

gave results larger than the use of all verticals. Except for flumes with vertical sides, however, only 7 per cent of the results were larger. In vertical-sided flumes one-third of the results were larger, so that while all experiments on flumes gave an average variation of 0.9 per cent, the mean of all variations was −0.6 per cent.

Table V indicates that in flumes or lined sections such as are usually used for canal-rating sections, the observation of velocities in from 12 to 20 verticals will give an increased accuracy of about 1 per cent over the results obtained with from 6 to 10 verticals, and about 3 per cent greater than with from 3 to 5 verticals. Under the most favorable conditions where the rating curve will remain fixed, the measurement of from 12 to 20 verticals, depending on the size of the section, may be warranted. Where the rating may be affected by channel changes during the season or under such conditions as are usually obtainable in the field, measurements based on from 6 to 10 verticals should represent good practice. The use of from 3 to 5 verticals will give results as closely as the rating curves derived can be applied to changing channel conditions in many cases and may be sufficiently close for some purposes. Using 8 verticals, only one-seventh of the results differed by more than 2 per cent; and using 4 verticals, only one-sixth differed by more than 5 per cent.

In the more irregular earth sections larger variations were found. This is to be expected, as in these the velocity and depths both change more rapidly near the sides than in the case of flumes. The use of an average of 8 verticals in earth sections gives results of similar accuracy to those obtained with only one-half as many verticals in flumes and lined sections. The use of an average of only 4 verticals gives results with average differences of nearly 9 per cent, and the variations of single experiments are much greater. It would appear that to obtain equal accuracy in gagings in earth sections with those secured in flumes about twice as many verticals should be observed. The number used will depend on the accuracy desired and the size of the canal. Less than from 6 to 8 verticals can not be recommended, and probably 8 to 12 would represent good practice. For more accurate work from 15 to 20 may be used, although where great accuracy is desired the measurements should be made in regular rating sections. Using 8 verticals, only one-tenth of the experiments differed by more than 5 per cent; using 4 verticals, one-third of the results differed by over 10 per cent.

A comparison of these results with those given for the different methods of observation of the velocity in the verticals can be made to determine the relative advantages of using either more verticals or taking more points in each vertical. The use of the 0.2- and 0.8-point method gave results averaging 0.7 per cent too high. The use of an average of 8 verticals in flumes and lined sections gave an average of 0.6 per cent too small. The use of 8 verticals obtained with the 0.2 and 0.8 method would tend to balance these errors, and in many cases might give as

accurate results as the more detailed observations. The use of the 0.6-point method gave results averaging 4.8 per cent too high, and the use of from 3 to 5 verticals in flumes and lined sections gives an average of 3 per cent too small. Apparently where few verticals are to be observed, the use of the 0.6-point method may be preferable, as the errors will tend to balance. This may be expressed by saying that about the same relative detail should be used in measuring the velocities in the verticals that is used in the number of verticals observed.

The results are obtained by using the verticals taken in the detailed measurements and selecting every alternate or every fourth vertical and computing the discharge that would have been obtained had only these verticals been observed. It is possible that gagings where the lower numbers of verticals were to be observed could be made to give closer results by using some means for the selection of the location in the canal section at which the verticals should be taken. It has been previously shown that the use of velocity measurements at the 0.2- and 0.8-depth points will give very nearly the same results as measurements at 6 or more points in the vertical and that a single observation at 0.6 gives results within 5 per cent of being correct.

If one or two points can be found in the vertical velocity curves the velocities of which can be used to determine the average velocity of the whole vertical, it would seem probable that perhaps 2 verticals on the horizontal velocity curve could be found which could be used to give the average velocity in the whole cross section. Such points, or index verticals, as they may be called, would be useful in the rougher measurements often needed in canal operation, and information as to the relative accuracy of such methods should be of value.

Two such selected verticals may be used to determine the discharge in two ways. In one the velocities only might be used and the cross-section area more carefully determined, if not known from previous observation. In the other the observed verticals may be used to obtain not only the mean velocity but also the depths at these verticals, and the width of the section may be used to determine the cross-sectional area.

The use of such index-vertical methods is, of course, most applicable to canal sections such as flumes which have practically uniform depths, as the error in determining the cross section is largely eliminated.

The measurements were examined to see whether such index verticals could be found. The horizontal velocities and cross sections were plotted on a sufficiently large scale so that the velocity and depth at any point could be read from the curves. Such index verticals would be most easily used if their distance from the sides is some definite proportion of the water-surface width. Verticals located at different points were tried. The different types of canal cross sections are discussed separately. The general results are given in Table VI.

TABLE VI.—*Discharge and velocity of various types of canals by measurements of two selected verticals*

Observations.	Number of observations.	Difference from correct discharge (per cent).			
		Average error of all observations.	Average variation of a single observation.	Extent of variations.	
				Plus.	Minus.
Total discharge in flumes with vertical sides:					
For points one-fifth from sides.....	22	+ 1.7	2.1	+ 4.5	− 1.4
For points one-sixth from sides.....		− 1.4	2.6	+ 5.1	− 6.5
Velocities only:					
Concrete-lined canal, steep side slopes—					
For points one-fifth from sides.	10	− 2.3	2.7	+ 2.0	− 5.7
For points one-fourth from sides		+ 1.3	3.1	+ 6.1	− 3.7
Concrete-lined canals, wide and flatly sloping sides—					
For points one-fifth from sides.	6	− 1.0	1.9	+ 1.6	− 5.3
For points one-fourth from sides		+ 1.4	1.8	+ 3.6	− 1.2
Average earth canals, sloping sides—					
For points one-fifth from sides.	15	− 2.3	5.8	+ 9.0	−13.6
For points one-fourth from sides		+ 4.3	5.2	+15.4	− 3.8
Average earth canals, steep sides—					
For points one-fifth from sides.	20	− 4.2	5.6	+ 7.2	−17.8
For points one-fourth from sides.		+ 4.9	5.7	+12.1	− 6.7
Earth canals, relatively deep sections—					
For points one-fifth from sides.	8	− 5.7	8.2	+10.0	−19.6
For points one-fourth from sides		+ 2.6	5.3	− 9.4	+13.8
Total discharges, points one-fifth from sides:					
Average earth canals, sloping sides..	15	+ 1.1	5.0	+14.6	− 8.2
Average earth canals, steep sides...	20	+ 3.3	6.0	+14.2	−10.4
Earth canals, relatively deep.....	8	+ 2.0	7.0	+14.1	−12.1

For vertical-sided flumes 22 gagings were available. The depths varied from 0.7 to 4.4 feet, the widths from 2 to 17.7 feet, and the discharges from 2 to 400 second-feet. The velocities and depths at points at a distance of one-fifth and one-sixth of the width from the sides were used to obtain discharges which were then compared with those obtained by the complete gaging. In such flumes with vertical sides the depths are practically uniform, and the use of the depth at only two points would cause little error in the resulting area. These results show that the two points whose mean velocities will equal that of the whole cross section lie generally between one-sixth and one-fifth of the width from the sides and that the error in using such index velocities at either proportion of the width averages about 2 per cent and does not exceed 5 per cent, except in a few cases.

For concrete-lined canals the canal section is uniform, and the cross-sectional area would be known for any depth. In such canals the discharge can be obtained from determinations of velocity and known areas for given depths. The comparisons given in Table VI are based on

velocity alone. The lined sections were subdivided into two classes: Those with relatively steep sides and those following the flatter slopes more usual to earth canals. There is no marked difference in the results of the two types. These measurements indicate, as was to be expected, that the points of mean velocity are farther from the water edges in sections with sloping sides than in the vertical-sided flumes and occur between one-fifth and one-fourth of the width of the water surface from the edges. The average and maximum errors are not large.

For earth canals results are given for both velocities and for total discharges. The results for such sections are more variable. The velocity at from one-fifth to one-fourth of the width from the edges will average to give results close to the actual velocity for the whole section, but individual gagings may vary from the mean by over 15 per cent. The results for the total discharge are more consistent than those for velocity alone. The error in the cross-sectional area, due to using the two measurements of depth to give the mean depths, tends to balance some of the errors in velocity. For all measurements the determination of the depth and velocity at points one-fifth of the width of the water surface from the sides gives average results from 1 to 3 per cent too high. Any single gaging will average to give errors of 5 to 7, and they may be as high as 15 per cent.

These results indicate that under favorable conditions two index verticals can be found in canals, the velocity at which will agree with the average for the whole cross section. These points are from one-fifth to one-sixth of the width of the canal from the sides in sections with vertical sides and from one-fourth to one-fifth for other types. In sections with vertical sides, such as flumes, and in earth sections the depths at these index verticals will also be quite close to the average depth in the whole section, so that the index points can be used also to determine the total discharge. In definite sections with sloping sides, such as concrete-lined canals, it is preferable to use known relations of depth and area and use the index points for the determination of velocities only.

Such short-cut methods would not generally be desirable at permanent rating stations. They might be useful for approximate measurements where time was an important factor, or as checks on the division of water in canal at large turnouts. Such gagings could be made of the canal above and below, and also of the turnout. Where other means of measuring or controlling the device are not available, such rapid methods might be of value.

SUMMARY

Comparisons of various methods of current-meter gaging of irrigation canals are made with measurements in which the velocities at from 70 to 120 points were taken. Canals of various types of cross section having discharges of from 2 to 2,600 second-feet and velocities of from 0.5 to 8.0 feet per second were included.

In 96 measurements the 0.2- and 0.8-depth, or two-point, method gave results averaging 0.73 per cent too high, and the 0.6-depth, or single-point, method gave results 4.80 per cent too high. The average variation for a single measurement was 1.5 per cent for the two-point method. If the results for the single-point method are corrected by −5 per cent, the average variation of a single observation is 2.5 per cent.

In 55 measurements the vertical integration method gave results averaging 0.76 per cent too high, and an average variation for a single observation of 2.07 per cent. The use of three-point methods gave errors greater than the two-point method alone.

There were no marked variations of the accuracy of any of these three methods due to difference in velocity, depth, or value of n in Kutter's formula.

In 92 measurements to determine the coefficient to be used to reduce the maximum surface velocity as measured by small floats to the mean for the entire cross section, the coefficient was found to vary with the value of n in Kutter's formula and the size of the canal. For water cross sections of over about 35 square feet the coefficient remains constant for any given value of n. A table is given for the coefficients for the range of conditions covered by the measurements. The coefficient varies from 0.60 to 0.91 for different conditions. The average variation of the coefficient for a single observation from the mean values was about 6 per cent, and in one-fifth of the observations exceeded 10 per cent.

In 89 experiments on the use of observations of varying numbers of verticals across the width of canals, it appears that in uniform cross sections, such as flumes or lined canals, observations in 8 verticals give an average within 1 per cent and in 4 verticals within 3 per cent of the discharge obtained with 16 verticals. In earth canals observations in 8 verticals give an average within 3 per cent and 4 verticals within about 9 per cent. For equivalent accuracy about twice as many verticals should be observed in ordinary earth sections as in uniform lined sections.

It was found that the use of only 2 verticals located from one-fifth to one-sixth of the width of the water surface from the sides of the section in canals with vertical sides such as flumes, gave results within an average of 2.5 per cent. In concrete-lined sections with sloping sides similar results were obtained where the velocities were measured at from one-fifth to one-fourth of the width from the sides, and the areas were secured from the known cross sections.

In earth canals 2 points from one-fifth to one-fourth of the width of the water surface from the sides give velocities varying from the mean of the whole cross section by about 6 per cent. Where the depths at these two points are used to give the average depth, the total discharge is determined with an average error of about 6 per cent. Errors in individual experiments were much higher.

RELATION OF SULPHUR COMPOUNDS TO PLANT NUTRITION

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INTRODUCTION

The four elements, nitrogen, phosphorus, potassium, and calcium, still play the most important rôle in soil treatment. For a number of years, however, other materials which stimulate growth in vegetation have been studied by chemists and agronomists.

The so-called catalytic fertilizers, such as the salts of manganese, have often been shown to increase plant growth. In addition, studies have been made of radium, lithium, sodium, arsenic, barium, copper, and some other elements. While these may stimulate plant growth, their application is not at present regarded as of economic importance. These elements are either not at all necessary for the plant's cycle of growth or, so far as we know, are abundantly supplied in all ordinary soils.

In the case of sulphur the relation appears to be somewhat different. It was pointed out in 1911 by Hart and Peterson (5)[1] that the total sulphur content of the soils examined was low, being approximately equal to the phosphorus content. This work has been confirmed by Shedd (12) for Kentucky soils and by Robinson (11) for the important soil types of the United States. It was further shown by Hart and Peterson (5) that the sulphur content of our common farm crops was considerable, cereal grains containing about half as much sulphur as phosphorus and legume hays sometimes more sulphur than phosphorus, while the Cruciferae, such as cabbage, turnips, etc., may contain two to three times as much sulphur as phosphorus.

It has been urged by Hopkins (6) that the high sulphur content of plants does not represent their needs, but merely shows the superabundance of sulphates in the soil water, with an extraordinary consumption by the plant. This may apply to the stem and roots of plants, but not to the seed. The seeds maintain a fairly constant composition and, as shown by Peterson (9), either contain but traces of sulphates, or more probably none at all. The criticism, then, that a high sulphur content of a plant merely represents a large soil supply can not possibly hold for seeds. It is true that the sulphate sulphur and probably other forms of sulphur in the stems and roots of plants will vary with the soil supply. In these plant parts sulphates may be present where the soil supply is plentiful. The same statement, however, is equally true of phosphates.

[1] Reference is made by number to "Literature cited," p. 249.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 6
Nov. 8, 1915
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Minimum requirements for maximum plant development have never been established for any of the essential elements. In addition, the demands for sulphur will be related to the character of the plant compounds elaborated by the different species of plants, even in the leafy portion. A cabbage crop that absorbs 100 pounds per acre of sulphur trioxid makes use of this material in a different way from a potato crop which absorbs but 11 pounds of sulphur trioxid. In the cabbage, sulphur compounds characteristic of the species are formed in abundance, thus creating a demand for a large sulphur supply. Alfalfa hay, constructed abundantly of protein compounds even in the stem and leaf, will demand and contain more sulphur than the low-nitrogen-containing residual straws of cereals. In either of the above cases used for illustration—namely, cabbage and alfalfa—it has been found that 30 to 50 per cent of the total sulphur may be present as sulphate sulphur. Nevertheless, this makes the total organic sulphur in an acre's growth of these crops very considerable—about 30 and 50 pounds of sulphur trioxid, respectively. In this connection let us again mention the fact that the annual rainfall will carry to an acre not more than 17 to 20 pounds of sulphur trioxid, while the loss by drainage may equal and even exceed this quantity. While we have no knowledge as to whether the excess of sulphates absorbed by the plant is of physiological importance, it is, nevertheless, clear that a supply of sulphur in this form in the plant indicates that the plant has not been limited in the elaboration of organic compounds for which sulphur is necessary. In fact, we suggest that information as to whether sulphur is a limiting factor for plant growth in any soil may probably be obtained by testing for the presence of sulphates in the plants grown on that soil. Their presence would indicate that there was a sufficient supply for all constructive purposes in which sulphur is involved.

From the facts presented on crop demand and soil supply we seem perfectly justified in including sulphur with nitrogen and phosphorus in the first group of essential elements which are limited in quantity in our common soils and in constant and relatively large demand by crops. On the same basis, potassium, calcium, and magnesium fall into a second group, while iron, constituting the third group, represents an element usually in abundance in soils and utilized in but small quantities by farm crops. Consequently, on the basis of total analysis and mathematics, sulphur should be of equal importance with phosphorus. Here, however, is where very probably total analysis and mathematics will not find complete justification for their use as the sole instruments in measuring permanent soil production. In collaboration with Prof. Fred (3), the senior author has pointed out the very great difference in the effect of phosphates and sulphates on important biochemical processes in the soil. In these studies it has been shown that soluble phosphates increase enormously the number of soil organ-

isms and the rate of ammonification and destruction of organic matter, while the sulphates activate but slightly in these directions. The processes mentioned are admitted to be of great importance to the plant's nutrition and environment, involving, as they must, not only a more rapid formation of readily soluble compounds of nitrogen and a possible destruction of harmful organic materials, but a greater saturation of the soil moisture with carbon dioxid, resulting in increased solution of mineral materials necessary for rapid growth.

While from the application of analytical chemistry and mathematics we should be led to give equal importance to phosphorus and sulphur in plant production, from their relation to important soil biochemical processes we must certainly ascribe to phosphorus the more important rôle. It has been demonstrated beyond question in certain phases of fermentology that cellular and enzymic activities are markedly increased by the presence of soluble phosphates. Harden and Young (4) have shown that the activation of the yeast cell or its zymase is greatly accelerated by the presence of these substances, and we now know that such activation by phosphates is not confined to the yeast plant but may also extend to the soil flora.

Consequently, in the case of phosphorus we have at least two factors operating to make it important in the soils—supply and physiological action; while in the case of sulphur the more important rôle will be merely as a source of supply. This, however, may not always be its only function, as will be shown later, where in the case of red clover it appears to have rather specific effects on root development; but besides such specific effects it appears at present that sulphur as sulphate in the soil serves essentially as the source of needed sulphur. It, therefore, in our judgment becomes important to accumulate information as to which agricultural plants will be affected by an increased concentration of sulphates in the soil water.

For some time sulphur in its elemental form has been used in the control of certain plant diseases. Incidental to this work there has accumulated much contradictory evidence relating to its effect on the crop yield. Opinion has been freely expressed as to how it acts in the soil, but with little definite agreement. In France especially, investigations have been active on the use of elemental sulphur with a large number of different plants. Work has been done with turnips, beans, celery, lettuce, potatoes, onions, spinach, and other crops. Various results have been obtained, but generally increased yields have been reported. Boullanger and Dugardin (1) place elemental sulphur among the catalytic fertilizers and have reported very favorable results from its use. They are of the opinion that its action is on the soil flora, in some way stimulating the breaking down of organic matter and ammonia production, although their observations show that it has quite a retarding action on nitrification. They further made the interesting observation

that in sterilized soil the addition of elemental sulphur had no effect in increasing plant growth, confirming their idea that elemental sulphur acted through some influence on the soil flora. Demolon (2) believes that sulphur not only acts by stimulating the soil flora but, in addition, acts as a source of needed sulphur after it has been oxidized in the soil. He showed conclusively that flowers of sulphur would gradually oxidize to sulphates in the soil, a statement which we have confirmed and which likewise has been shown by Lint (8) to be true. The fact that elemental sulphur is oxidized in the soil probably has direct bearing on the necessity for the use or presence of adequate quantities of lime or other basic material in a soil receiving this treatment. This may not apply to all crops, but might properly explain the results secured by Wheeler, Hartwell, and Moore (16), who showed that there was injury to cereals following the application of elemental sulphur for the prevention of potato scab, unless a considerable quantity of lime had been used in the soil. From the South Oregon Experiment Station, Reimer (10) reported large increases in the yield of alfalfa by the direct use of elemental sulphur. Whether these experiments were conducted on soils of high basicity has not been reported.

The possibility of injury to the crop by partial oxidation of the elemental sulphur to sulphite must always be kept in mind. Thalau (15) has shown that sulphites of ammonium and calcium are toxic to plants in dilute solution, but probably are not so toxic in the soil itself. The fate of the elemental sulphur introduced into a soil will ultimately be its oxidation to a sulphate, but the formation of intermediate compounds and their toxic effect may account for the contradictory results that have been recorded from its use. For example, Janicaud, Hiltner, and Gronover (7) report deleterious effects with tomatoes from the use of elemental sulphur, and some of the results of Sherbakoff (14) in the treatment of potatoes for scab are of a similar order. Consequently, the attempted introduction of elemental sulphur as a source of sulphur in plant nutrition should, in our judgment, be viewed with caution.

The basis for this statement will be amplified in the following report of experimental work. After this manuscript had been prepared, the work of Shedd (13), of the Kentucky Agricultural Experiment Station, was made public. In this work use was made of a number of sulphates and sulphids, and of elemental sulphur. Good results from the use of a number of these materials are reported. Elemental sulphur and gypsum were helpful to tobacco, and elemental sulphur was materially beneficial to turnips on the soil investigated. Clover on this soil was not helped by sulphur-containing fertilizers, with the exception of a benefit from the use of potassium sulphate. Other plants, such as mustard, cabbage, and radish, showed increased growth with sulphur-containing materials.

EXPERIMENTAL WORK

Beginning in 1911, experiments have been conducted in the greenhouse to determine the influence of sulphates and sulphur on the growth of some common farm crops. Seven different crops representing three different orders have been included in the work up to the present time. They were distributed by orders as follows: Cruciferae—radish (*Raphanus sativus*), rape (*Brassica napus*); Gramineae—oats (*Avena sativa*); barley (*Hordeum vulgare*); Leguminosae—red clover (*Trifolium pratense*), bean (*Phaseolus vulgaris*), pea (*Pisum sativum*). It should be said of plants grown in this way that they sometimes do not develop so well as under field conditions. The lessened light of winter as compared with summer, for example, retards growth, and in the early fall and late spring the day temperatures are likely to become excessive. Also, possibly owing to the protection from wind and the absence of insects, the plants rarely seed well. Despite these influences, however, our crops have grown well in most cases and in some cases have developed luxuriantly. It is true, moreover, that in all cases the effect of varying fertilizer treatments is reliable for comparison, since each crop, save the food supply, was grown under conditions as uniform as possible.

METHOD OF INVESTIGATION

The soil used in this work was the Miami silt loam which predominates on the University Hill Farm. It was obtained by removing the surface vegetation and selecting the surface soil to a depth of about 4 inches. This material was then sifted through a ¼-inch screen and thoroughly mixed. There was practically no loss in the sifting, as hardly a stone was found and the sifted product was smooth and of excellent quality.

A total analysis of the soil showed the following composition, based on the dry matter: Nitrogen (N), 0.15 per cent; phosphorus pentoxid (P_2O_5), 0.14 per cent; sulphur trioxid (SO_3), 0.04 per cent; calcium carbonate ($CaCO_3$), 0.33 per cent; humus, 1.38 per cent.

The humus was determined by the official methods of analysis of the Association of Official Chemists.[1] Fifteen kilos (33 pounds) of this soil were placed in rectangular cypress boxes 16 inches long, 14 inches wide, and 5 inches deep. Seven different fertilizer treatments were tried in duplicate boxes of the soil, as follows:

Boxes Nos.		
1–2.	Control (no fertilizer).	
3–4.	Complete fertilizer:	Gm.
	Tricalcium phosphate ($Ca_3(PO_4)_2$)	12.0
	Potassium chlorid (KCl)	4.5
	Sodium nitrate ($NaNO_3$)	10.0

[1] Wiley, H. W., et. al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

Boxes Nos.		Gm.
5–6.	Complete fertilizer+sodium sulphate (Na_2SO_4)	12
7–8.	Complete fertilizer+calcium sulphate ($CaSO_4$)	12
9–10.	Sodium sulphate (Na_2SO_4)	12
11–12.	Calcium sulphate ($CaSO_4$)	12
13–14.	Sulphur (flowers)	5

All of these materials were mixed with the soil at the beginning of the experiments, except the sodium nitrate. This was applied in solution in three separate portions as the plants developed. Sulphur was not included in the treatment of the earlier experiments. These amounts of fertilizer are equivalent to the following applications per acre to the surface 8 inches of soil, assumed to weigh 2,000,000 pounds: Tricalcium phosphate, calcium sulphate, and sodium sulphate, 1,600 pounds each; potassium chlorid, 600 pounds; sodium nitrate, 1,330 pounds; and sulphur, 665 pounds.

While these applications may appear excessive as compared with field applications, nevertheless it should be remembered that in these experiments there was a thorough and complete mixing with the entire soil mass. In some cases the soil was limed. For this purpose 10 gm. of calcium carbonate were added to each box in the set. This was at the rate of 1,330 pounds per acre of a depth of 8 inches.

Except in the case of large seeds, such as beans and peas, the seeds were sown liberally in four rows across the boxes and thinned when well developed to 16 plants per box. The larger seeds were germinated on paraffined mosquito netting stretched over distilled water, and transplanted to the soil when well developed. The usual care was taken to support the taller crops and suppress development of fungi and insects, but the use of any sulphur-containing sprays was of course carefully avoided.

When the crops were mature, they were harvested and weighed while fresh. They were then dried quickly in steam-heated trays at about 50° C. and allowed to stand exposed to the air from two to three weeks to become air-dried, in which condition they were finally weighed.

The final comparative weights will be presented in the following tables, in which the weights given are averages obtained from duplicate boxes. In some cases, as indicated, the seed has been separated from the straw and weighed separately. Owing to the difficulty in recovering the roots from the soil, they have been neglected in most cases.

LEGUMINOSAE

BEANS (*Phaseolus vulgaris*).—The variety of beans grown was Davis White Wax. In crop A only 10 plants were grown per box. This crop followed two successive crops of clover on the same soil, the first crop of clover having been fertilized. Crop A was fertilized as usual, except that no sulphur was added to boxes 13 and 14. Crop B was not fertilized. Crop C was completely fertilized. Crop D was grown on a

different set of the same type of soil, but which had produced two crops of rape (both fertilized) and three crops of radishes, the last radish crop having been fertilized. The soils were limed for this crop. The yields of air-dried crops are given in Table I.

TABLE I.—*Average weights (in grams) of air-dried bean crops*

Treatment.	Seed.					Straw and pods.				
	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops.	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops.
1. Control	6.4	0.7	7.1	5.3	100	15.7	12.4	34.7	26.8	100
2. Complete fertilizer	8.0	5.7	13.8	15.9	223—	21.41	19.1	42.5	45.8	144
3. Complete fertilizer + sodium sulphate.	6.9	3.4	12.9	12.8	185	18.8	20.1	46.8	43.3	144
4. Complete fertilizer + calcium sulphate.	10.4	6.3	17.3	10.1	226—	24.4	22.3	44.3	40.8	147
5. Sodium sulphate only	7.1	5.9	13.3	6.6	169	19.2	17.1	31.7	26.0	105
6. Calcium sulphate only	6.6	6.1	11.7	4.6	149	14.8	20.2	31.8	21.5	89
7. Sulphur only	3.0	4.1	1.9	0.9	51	17.9	20.3	25.3	19.3	92

The relative yields of seed showed irregular results from the application of the sulphates. When added to the usual complete-fertilizer ration, sodium sulphate depressed growth, while calcium sulphate slightly favored it. When applied alone, both salts gave results decidedly better than the control untreated soils. In this case the soluble sodium sulphate gave better results than the comparatively insoluble calcium sulphate. It seems possible that the superior results from the sodium sulphate applied alone as compared with its effect when added to the complete-fertilizer treatment may have been due to an unfavorable excessive accumulation of soluble salts in the latter case which might not occur when it was added alone.

The relative yields of straw from this crop showed no significant effects which might be due to the added sulphates. Sulphur alone was decidedly injurious to the beans. The effect is more noticeable in the case of the grain than with the straw. This might be expected to obtain, since the plants already weakened in general vitality would probably be depressed in the power of reproduction. This was more probably due to sulphites and other toxic oxidation products of the sulphur than to the sulphur itself. It could not be due merely to the acidity of the soil produced by oxidation of the sulphur, for it occurred with crop D, which was limed.

CLOVER (*Trifolium pratense*).—The variety grown was Medium Red. Crop A was grown on fresh fertilized and limed soil. Crop B followed crop A on the same soil without fertilizer treatment, but with the addition of fresh soil in boxes 13 and 14, to which calcium carbonate and elemental sulphur were applied. Crop C was grown on completely

renewed unlimed soil with the usual complete-fertilizer treatment. Crop D was grown on soil which had borne two successive fertilized crops of rape and two successive crops of turnips (*Brassica napus*), the last crop of turnips only receiving fertilizer. This clover crop was limed and fertilized. All the crops were allowed to reach the late-blooming stage, but they failed to produce seed. The roots of crops B and C were separated as carefully as possible from the soil and weighed separately from the tops. The yields of air-dried matter are given in Table II.

TABLE II.—*Average weights (in grams) of air-dried clover crops*

Treatment.	Hay.					Roots.				
	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of all crops.	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops B and C.
1. Control	31.8	56.2	11.7	92.0	100	.....	49.5	16.8	.....	100
2. Complete fertilizer	45.3	71.5	48.1	95.0	136	.....	48.5	37.4	.....	130
3. Complete fertilizer+sodium sulphate	54.8	72.2	67.0	99.8	153	.....	41.4	31.9	.....	111
4. Complete fertilizer+calcium sulphate	46.0	79.2	73.7	108.2	160	.....	48.8	36.4	.....	129
5. Sodium sulphate only	33.0	65.9	23.6	93.9	113	.....	67.7	33.1	.....	152
6. Calcium sulphate only	27.8	62.5	29.0	116.4	123	.....	92.9	31.9	.....	188
7. Sulphur only	.....	49.1	23.6	.....	38	.....	71.9	21.5	.....	141

In the yield of hay there was no doubt about a marked stimulating effect of both sulphates upon growth. Stimulation was equally evident when they were added to the complete-fertilizer treatment and when they were applied alone. In both cases the best results were produced by the less soluble calcium sulphate. Elemental sulphur had a very depressing effect. The average yield from this treatment was but little more than one-third the yield from the control, and in crop D the clover entirely failed to grow where elemental sulphur was applied. Plate XX, figure 1, illustrates the influence of sulphates on the growth of clover.

Root development from the complete-fertilizer treatment was depressed somewhat when sodium sulphate was also applied, but was unaffected when the calcium sulphate was added. We are inclined to ascribe this difference to the depressing effect of the more concentrated soil solution where the soluble sulphate was applied. The effect of the sulphates applied alone was very striking. In Plate XXI is shown the remarkable difference of root development from the different fertilizer treatments. From our limited amount of data calcium sulphate appears to be somewhat more active than sodium sulphate in producing this effect. In any case it appears that in this soil a sulphate has specific effects on the root development of this species. This may properly explain the oftentimes beneficial effects observed in the application of land plaster

to clover. While the form of the root system developed under the two treatments may not involve a larger feeding surface in the one case as compared with the other, yet it does seem very probable that the long root system developed where sulphate concentration was larger would favor that plant in times of limited water supply. The unavoidable conclusion from the results with red clover is that the reenforcement of the limited soil supplies of sulphur compounds by sulphates of sodium and calcium was decidedly beneficial to this crop.

PEAS (*Pisum sativum*).—The variety grown was Little Gem, a dwarf variety. Strong seedlings were transplanted to the soil six days after they were placed on the germinator. The soils had already produced two crops of clover and three of beans, the first crop of clover and the first and last crops of beans having been fertilized. Both clover crops had been limed. No elemental sulphur was added to box 13 and 14 for the first crops of beans. The data of the pea crop are given in Table III.

TABLE III.—*Average weights (in grams) of the air-dried pea crop*

Treatment.	Seed.	Straw and pods.	Relative yields of seeds.	Relative yields of straw.
1. Control	0.18	4.42	100	100
2. Complete fertilizer	.21	3.99	117	90
3. Complete fertilizer+sodium sulphate	.24	4.12	133	93
4. Complete fertilizer+calcium sulphate	.97	4.54	539	103
5. Sodium sulphate only	.60	4.41	333	100
6. Calcium sulphate only	.82	3.84	456	87
7. Sulphur only	.03	2.47	17	56

This crop did not grow vigorously, and the differences of yields have, therefore, less significance than with the preceding crops. However, the increased yields of seeds where sulphates were added is surely remarkable. This is especially true for the calcium sulphate, both when added to the complete fertilizer and when added alone. Both sulphates when applied alone gave remarkable increases over the control soils. Sulphur alone was much more toxic than was the case with the crops already described. The straw shows no very great differences of yields, except where sulphur alone was applied. Here the depressing effect was somewhat less than in the case of the other leguminous crops.

Probably the negative effect of fertilizers upon the growth of straw on this crop should be attributed to the fact that the soils had been excessively cropped and fertilized. This would tend, on the one hand, to exhaust the control soil and, on the other hand, to render the fertilized soils too concentrated in soluble salts for good growth. Hence, the development was even poorer in some cases than the control. Apparently the sulphates especially favored the development of seed in this weakened crop. That such was not the case where sodium sulphate

was added to the complete fertilizer may have been due, as suggested for the previous crops, to a depressing effect of an excess of soluble salts. The favorable effects of calcium sulphate were most decided.

Summarizing the results obtained with the leguminous plants, it may be stated that sulphates added to this soil were decidedly beneficial to the growth of the crops so far investigated. With the large-seeded bean and pea the effects are practically confined to the increased seed development. With the hay crop, however, the results are favorable to the growth of the straw portion of the plant. Calcium sulphate in general is considerably superior to sodium sulphate in its fertilizing action. In the case of clover both of these compounds, when added separately, increased the root development markedly. This would tend to increase the feeding power of the plant and may largely account for the increase of hay produced by their use. Sulphur alone depresses the general development of the plant, with the apparent exception of the clover roots.

CRUCIFERAE

RADISHES (*Raphanus sativus*).—The variety grown was Earliest Scarlet Turnip. Crop A followed two crops of rape on the same soil, both of which had been fertilized. Crop A was not fertilized. Crop B followed crop A on the same soil and was not fertilized. Crop C was also grown on the same soils, but was fertilized. Fifty days from planting crop A, alternate rows of the crop were harvested from one set of boxes for photographing. These were dried and the weights recorded. The remaining plants were allowed to develop seed and the residue rejected. Plate XX, figure 2, is therefore the only available comparison covering the whole crop. The air-dried yields are given in Table IV. (See Pl. XX, fig. 3.)

TABLE IV.—*Average weights (in grams) of air-dried radish crops*

Treatment.	Crop A.		Crop B (whole plants).	Crop C (whole plants).	Average relative yields of whole plants for all crops.
	Tops.	Roots.			
1. Control	0.2	2.5	19.9	10.3	100
2. Complete fertilizer	1.5	4.7	36.5	34.9	236
3. Complete fertilizer+sodium sulphate	1.2	4.7	30.5	48.0	256
4. Complete fertilizer+calcium sulphate	1.7	7.0	28.4	47.6	257
5. Sodium sulphate only	1.5	5.0	24.3	10.9	126
6. Calcium sulphate only	1.0	4.7	21.0	11.3	115
7. Sulphur only	.8	3.7	18.2	7.1	60

The results call for special comment. They show, especially where freshly fertilized (crop C), an unmistakable stimulus to growth by sulphates. The effect is much more pronounced where the sulphates were applied alone than where the complete-fertilizer ration was used. A

point of special interest in these results is the fact that sodium sulphate gave quite as good results as calcium sulphate when added to the complete-fertilizer ration. This suggests that we were dealing here with a plant more tolerant of the concentrated soil solution than were the legumes grown. The radish was also more tolerant of elemental sulphur than were any of the legumes, although the growth in its presence was somewhat inferior to that of the control plants.

Rape (*Brassica napus*).—The variety grown was Dwarf Essex. Crop A was grown on the usual soil, fresh and completely fertilized except for elemental sulphur. Crop B followed crop A on the same soil. The soil was refertilized and boxes with elemental-sulphur treatment were added. Crop C was grown on fresh-fertilized soil. Crop D followed crop C on the same soil and with the same fertilizer applications. The rape crops were harvested when the death of the basal leaves indicated the near approach of maturity. Data of the weights of the air-dried rape crops are given in Table V.

Table V.—*Average weight (in grams) of air-dried rape crops*

Treatment.	Tops.					Roots.				
	Crop A.	Crop B.	Crop C.	Crop D.	Relative weights with control = 100.	Crop A.	Crop B.	Crop C.	Crop D.	Relative weights with control = 100.
1. Control	54.0	12.7	11.6	15.3	100	8.5	2.0	2.7	.....	100
2. Complete fertilizer	80.5	29.0	36.4	27.7	188	11.8	3.8	5.1	.....	157
3. Complete fertilizer+sodium sulphate	90.0	30.9	45.6	40.9	222	12.3	2.6	5.3	.....	154
4. Complete fertilizer+calcium sulphate	78.5	32.9	45.4	50.0	221	12.5	4.9	6.3	.....	181
5. Sodium sulphate alone	59.5	13.9	15.8	14.3	111	8.5	2.3	2.8	.....	104
6. Calcium sulphate alone	57.0	14.7	13.5	13.3	105	8.8	3.0	3.3	.....	115
7. Sulphur alone	.....	13.6	12.3	4.2	32	.....	3.1	2.6	.....	44

It is clearly evident that the addition of sulphates benefited this crop, but especially so where they supplemented the complete-fertilizer ration. Apparently the demands for sulphur of the higher yields of tops from the fertilized plants accentuated the benefits from the sulphates in this case (Pl. XXII, fig. 1).

The sulphates of calcium and of sodium were equally efficient for rape. In the case of the roots only the calcium sulphate gave beneficial results. Possibly the soluble sodium sulphates increased the concentration of the soil solution to such an extent as to retard the growth of the roots. It is well known that in water cultures the roots of plants are more sensitive than the tops to such changes in the nutrient medium. As in water cultures, so, too, in these soil cultures, it appears that the growth of tops and of roots does not proceed parallel.

Rape was also grown upon sand. The sand employed was obtained from the Wausau Quartz Co., Wausau, Wis. It was an angular product, designated as No. 2, which passed almost completely through a sieve of 40 meshes to the inch, but was half retained by a 60-mesh sieve. It contained small amounts of impurities, but no sulphates. Fifteen kgm. (33 pounds) of this sand were placed in the usual boxes with the following fertilizer treatments:

Boxes Nos.		Gm.
1–2.	Tricalcium phosphate ($Ca_3(PO_4)_2$)	12.0
	Potassium chlorid (KCl)	4.5
	Magnesium nitrate ($Mg(NO_3)_2$)	2.5
	Sodium nitrate ($NaNO_3$)	8.0
	Calcium carbonate ($CaCO_3$)	5.0
	Iron chlorid ($FeCl_3$)	1.0
3–4.	Like 1 and 2+calcium sulphate ($CaSO_4$)	12.0
5–6.	Like 1 and 2+sodium sulphate (Na_2SO_4)	12.0
7–8.	Like 1 and 2+sodium sulphate (Na_2SO_4)	6.0

All of the salts, except sodium nitrate, were mixed with the sand before planting, but this was applied to the growing plants in portions from time to time. At 84 days of growth, when the plants gave the usual signs of maturity, the crop was harvested. The yields of the air-dried rape crops are given in Table VI.

TABLE VI.—*Average weights (in grams) of air-dried rape crops*

Treatment.	Tops.	Roots.	Relative yields when complete fertilizer=100.	
			Tops.	Roots.
1. Complete fertilizer	39.0	4.5	100	100
2. Complete fertilizer+calcium sulphate	43.0	10.0	110	222
3. Complete fertilizer+sodium sulphate	26.5	3.2	68	71
4. Complete fertilizer+½ sodium sulphate	31.0	3.5	80	78

In these cultures the calcium sulphate was beneficial, but the sodium sulphate depressed the yields as compared with the basal complete fertilization. The data show this effect of the sodium sulphate least where the smaller amount of salt was applied. This again seems to indicate that the depressed effect was due, in part at least, to an excessive concentration of soluble salts. If such an effect were appreciable, one would expect it to be more pronounced in the case of the sand than with soil on account of the lower absorptive power of the former, and such was the case. The calcium sulphate exerted a remarkable effect on the development of the rape roots in these cultures. An objection might possibly be raised that the beneficial effects upon root growth apparent with the soil cultures may have been due to imperfect separation of the

finer parts of the root system from the soil. Such objection would not apply to the sand cultures, which therefore gave conclusive evidence of the stimulating effect of calcium sulphate upon the root development of rape. The benefit to the tops from this salt was much less pronounced, but nevertheless definite. As in most other cases, the elemental sulphur was detrimental to the plants, presumably because of toxic action. There seems to be no doubt that the rape plant has specific need for sulphur, which should be met by including sulphates in its fertilizer treatment.

GRAMINEAE

BARLEY (*Hordeum vulgare*).—One crop was grown upon a set of soils which had already produced one crop of peas with fertilizer treatment and a second crop without fertilizer. The barley crop was not fertilized, as the pea crops had been light. The variety planted was New Zealand Chevalier. In Table VII are given the average air-dried weights of the yields from duplicate boxes.

TABLE XVII.—*Average weights (in grams) of air-dried barley crop*

Treatment.	Straw.		Grain.	
	Weight.	Relative yields when control=100.	Weight.	Relative yields when control=100.
1. Control	36.5	100	9.5	100
2. Complete fertilizer	59.0	162	10.5	111
3. Complete fertilizer+sodium sulphate	67.0	184	14.5	153
4. Complete fertilizer+calcium sulphate	62.5	171	15.0	158
5. Sodium sulphate only	43.5	119	14.0	147
6. Calcium sulphate only	38.5	106	17.0	179
7. Sulphur only	39.0	107	13.5	142

The limited data available are insufficient for the deduction of definite conclusions concerning the effects of the sulphur supply upon the growth of the barley crop. They indicate, however, that sulphur and the sulphates here applied had little influence upon the production of straw in this crop either when added to a complete-fertilizer ration or when applied alone. Conditions were decidedly different in the case of the grain. While the production of straw seems to have been limited, this amount of straw produced 40 to 80 per cent more grain in the crops receiving sulphur and sulphates alone than in the control crops. Likewise, the crops receiving sulphates in addition to a complete-fertilizer ration produced about 40 per cent more grain than those receiving only the complete ration (Pl. XXII, fig. 2).

OATS (*Avena sativa*).—This crop was grown upon a set of soils which had borne two unsatisfactory barley crops, the first of which had been fertilized. The oat crop was not fertilized. Wisconsin Wonder was

the variety planted. Unlike the barley, this grain crop showed decided differences in development upon the different rations during its growth, as shown in Plate XXII, figure 3. In Table VIII are given the average yields of the thrashed crop in the usual manner, the husks being carefully removed from the seed.

TABLE VIII.—*Average weights (in grams) of the air-dried oat crop*

Treatment.	Straw.		Grain.	
	Weight.	Relative yields when control=100.	Weight.	Relative yields when control=100.
1. Control	28.5	100	2.5	100
2. Complete fertilizer	56.0	197	5.0	200
3. Complete fertilizer+sodium sulphate	57.5	202	8.5	340
4. Complete fertilizer+calcium sulphate	54.5	191	8.5	340
5. Sodium sulphate only	19.5	68	2.5	100
6. Calcium sulphate only	19.0	67	2.5	100
7. Sulphur only	23.5	82	3.5	140

The statements previously applied to the limited amount of data on barley also apply to the oats. So far as the preceding table is concerned, however, it indicates, as in the case of barley, no appreciable effect of sulphates upon the development of straw when they supplement the usual complete-fertilizer ration. Sulphur and sulphates alone even depressed the yield of straw as compared with the control crops.

In the case of the grain, the application of sulphur and sulphates alone did not increase the yield as compared with the controls, although it increased the ratio of grain to straw. The crops receiving complete fertilizer indicate a marked stimulating effect of sulphates upon seed production in this crop. Those crops receiving sulphates in addition to a complete fertilizer produced 70 per cent more seed than those receiving complete fertilizer only.

The data from these two crops of the Gramineae family have shown a marked response of these plants to the application of sulphates by increased seed production. From these records it appears that under present common methods of fertilization these grain crops may frequently reach a maximum production of straw, but that the capacity of this yield of straw to produce seed may be greatly enhanced by the addition of calcium sulphate or sodium sulphate to the so-termed complete-fertilizer ration. In future investigations the writers plan to determine whether the indications here obtained with the Gramineae express a general and fundamental sulphur requirement of this family of plants.

The influence of the concentration of the soil sulphates on the sulphur content of plants has already received consideration (9), but it will not

be out of place to include further data on that subject. Work has been done especially on clover and rape. Data illustrating this influence are given in Table IX. The crops were air-dried.

TABLE IX.—*Influence of supply of sulphates on the sulphur and potassium content of clover and rape*

Treatment.	Clover tops.							Rape.					
	Crop B.			Crop E.				Crop B.			Crop D.		
	Sulphur.	Crop.	Quantity of sulphur removed.	Sulphur.	Crop.	Quantity of sulphur removed.	Potassium.	Sulphur.	Crop.	Quantity of sulphur removed.	Sulphur.	Crop.	Quantity of sulphur removed.
	Pr. ct.	*Gm.*	*Gm.*	*Pr. ct.*	*Gm.*	*Gm.*	*Pr. ct.*	*Pr. ct.*	*Gm.*	*Gm.*	*Pr. ct.*	*Gm.*	*Gm.*
1. Control	0.15	56	0.084	0.20	28	0.056	1.58	0.60	12	0.072	0.22	15	0.033
2. Complete fertilizer	.20	71	.142	.14	85	.119	2.42	.18	29	.054	.22	27	.059
3. Complete fertilizer + sodium sulphate	.20	72	.144	.21	99	.207	2.63	.87	31	.269	.78	41	.319
4. Complete fertilizer + calcium sulphate	.20	79	.558	.25	110	.275	2.31	.90	33	.290	.70	50	.350
5. Sodium sulphate only	.11	66	.072	.13	56	.072	1.64	1.18	14	.165	1.08	15	.162
6. Calcium sulphate only	.16	63	.100	.25	61	.152	1.36	1.18	14	.165	.90	13	.117
7. Sulphur only	.19	49	.093	.22	45	.099	1.32	1.00	13	.130	1.66	4	.066

As has been pointed out, the effect of a more concentrated soil-sulphur solution is to increase the total sulphur content of the root and the stem, but not of the seed. This influence is particularly great in the case of the leafy plant like the rape, but is not so marked in the red clover. In the rape the percentage variation of sulphur ranged from 0.20 to 1, depending upon the supply, while in the clover the range was from 0.10 to 0.20. In the case of one crop of clover there is included the total potassium content of this crop. It has been common, since the time of Boussangault, to explain the action of calcium sulphate in the soil as a liberator of potassium, and its effect as indirect. This explanation might still be used for our own results where calcium sulphate was added alone. In this case the growth of crop was so much increased over the growth in the control that the total potassium removed was considerably more than in the control. But where the complete fertilizer containing potassium chlorid is compared with the complete fertilizer plus calcium sulphate, such an explanation for the action of calcium sulphate becomes untenable. The increased growth due to the calcium sulphate in the presence of a complete fertilizer containing potassium can have no other explanation than that its action was direct rather than indirect.

SUMMARY

The data presented from these greenhouse studies with one type of soil indicate that certain plants are measurably increased in their growth by the addition of sulphates. We have emphasized in another

place the fact that sulphates have very little effect as compared with soluble phosphates on the soil flora. This difference in action will remove the sulphates from the category of effective fertilizers for all crops. Nevertheless, for certain plants and types of soil they will be beneficial if their only action is as a source of sulphur.

The plants most affected were the members of the Leguminosae and Cruciferae. It is probable that we should expect these classes of plants to be more responsive to the higher concentration of sulphates in the soil water than, for example, the Gramineae, owing to the higher protein content of the first group and the special sulphur-bearing bodies abundantly formed in the second group. In this soil, however, there was noticeable stimulation to seed production in both barley and oats, although there was little or no effect on the development of the quantities of straw.

In the case of clover the increase in air-dried matter due to calcium sulphate alone was about 23 per cent. With rape the greatest increase occurred where the calcium sulphate was superimposed upon a complete fertilizer, giving an increase of 17 per cent over the complete fertilizer. A similar order of increase was likewise observed with the radish crop, where the increase above a complete fertilization, due to the calcium sulphate addition, averaged 9 per cent.

In general, the calcium sulphate was more effective than the more soluble sodium sulphate. The special influence of sulphates on root development is pointed out. They were particularly effective with red clover and rape. In the case of red clover, which was more especially studied, the roots were much elongated where sulphates entered into the ration. This must result in a more extended feeding area for the plant and, in addition, increase its ability to withstand periods of drought.

The somewhat common observation of the benefit of land plaster to this plant can probably be closely correlated with this special effect of sulphates on root development, as well as its high protein character, which would make special demands for sulphur.

Whether recorded failures in the use of land plaster are to be correlated with wet seasons, a high sulphur content normal to the soil under observation, or the variety of plants used is a matter for future observation.

In these greenhouse experiments elemental sulphur was generally harmful. These harmful results occurred even in the presence of a generous supply of calcium carbonate. These results indicate that elemental sulphur may be toxic through its incomplete oxidation to sulphites; toxicity may also arise in the absence of sufficient basic material through the development of acidity from sulphuric acid.

Application of these results to field practice is reserved until more data on field plots are available.

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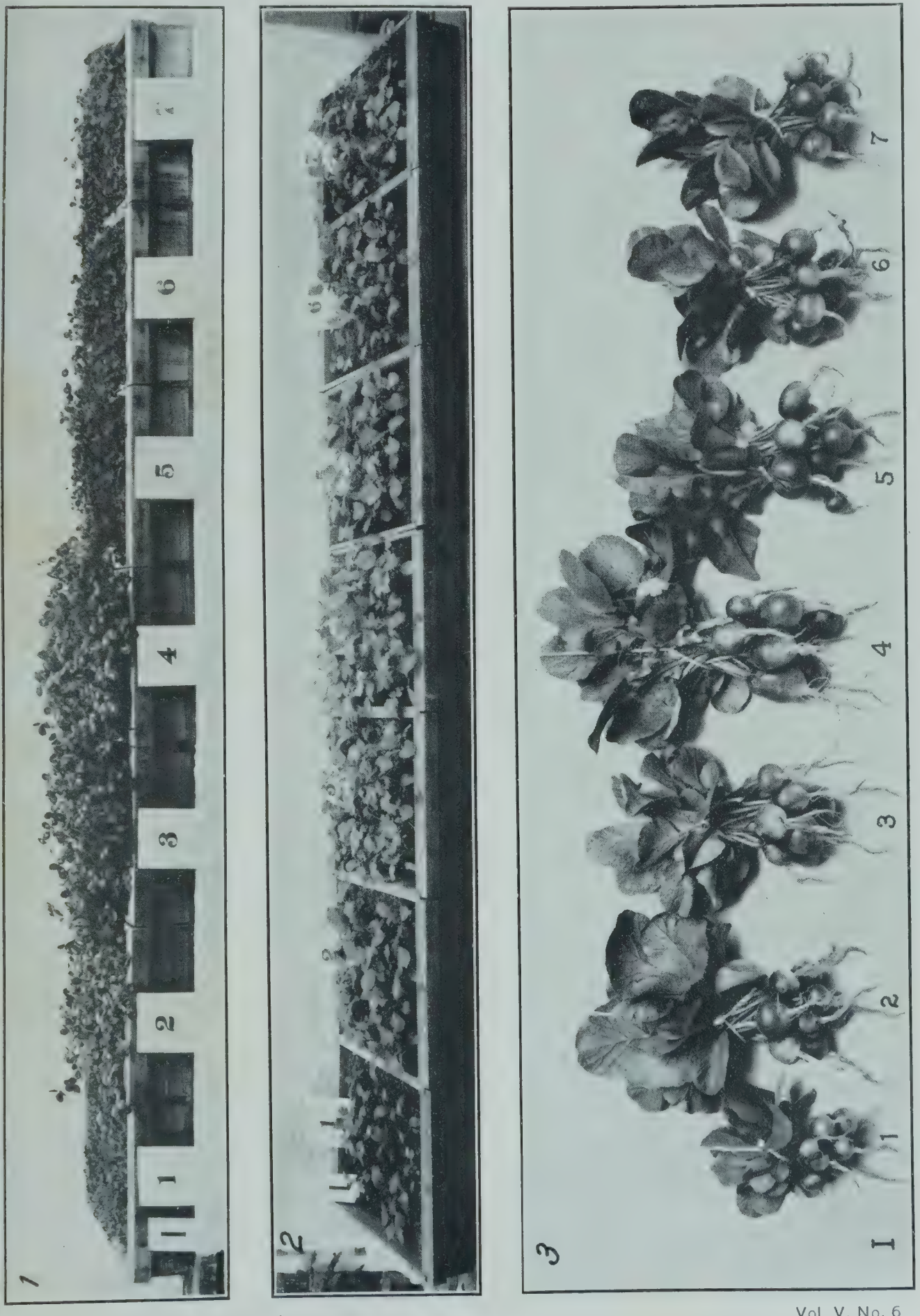
PLATE XX

Fig. 1.—Clover plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 2.—Radish plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 3.—Radish plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.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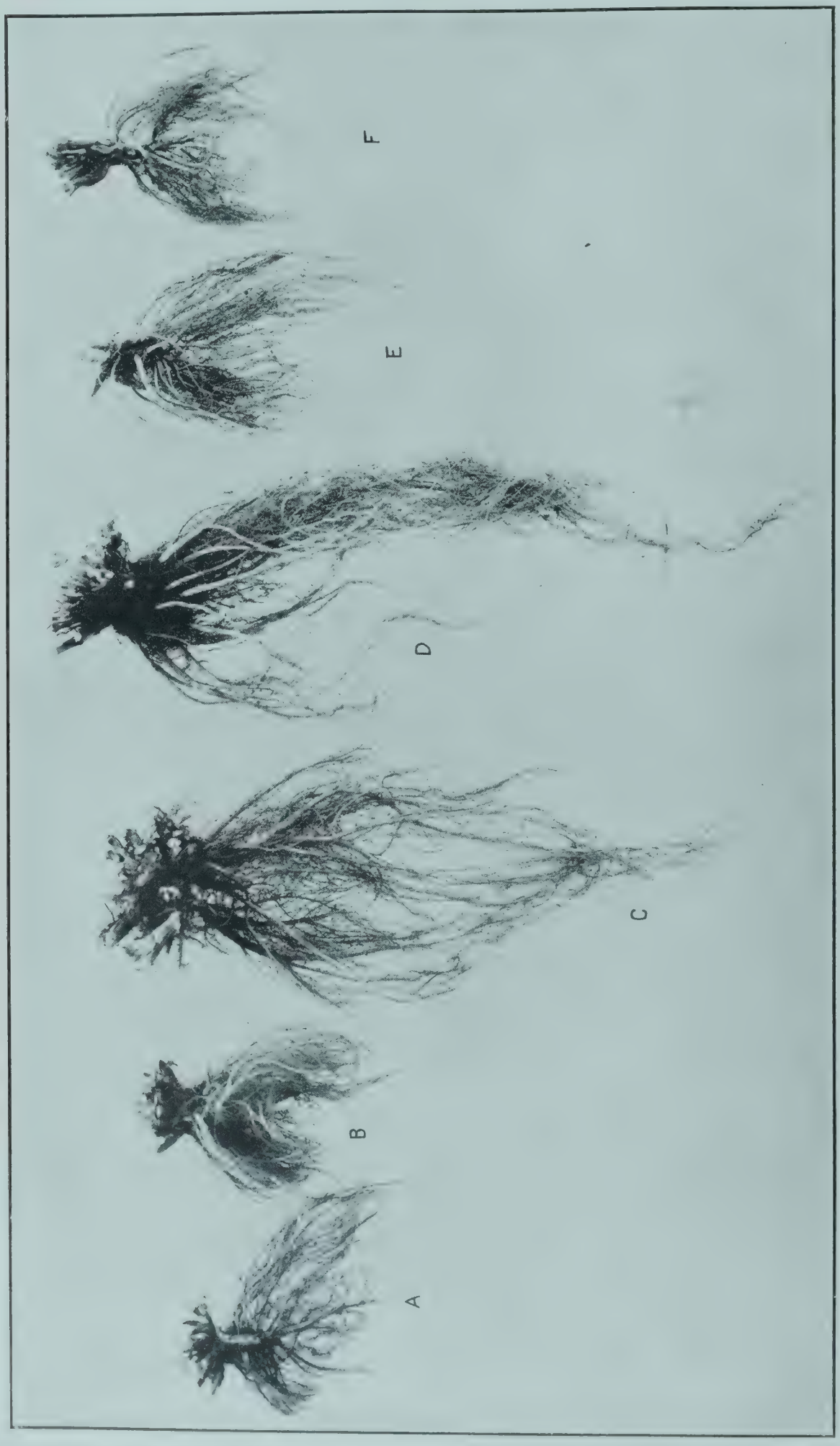
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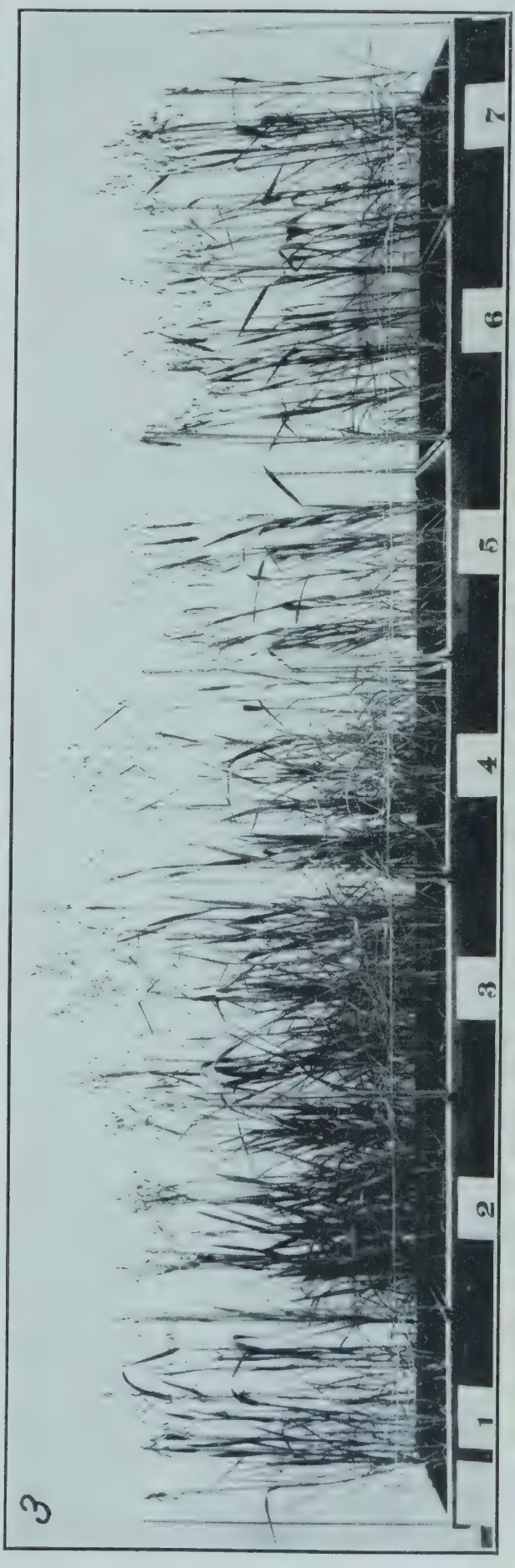

DISTRIBUTION OF THE VIRUS OF THE MOSAIC DISEASE IN CAPSULES, FILAMENTS, ANTHERS, AND PISTILS OF AFFECTED TOBACCO PLANTS

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Embryonic transmission of the mosaic disease from parent to offspring has not been observed in tobacco plants. Although the disease sometimes appears particularly malignant, so that normal capsule development is almost completely inhibited and few viable seed are produced, plants from such seed are healthy. The normal reproductive vigor of tobacco plants may not be seriously checked by the mosaic disease, especially if it makes its appearance late in the development of the plant. In such plants a nearly normal vegetative development has been attained and subsequent flowering and seed production appear to be little, if at all, inhibited.

It is of considerable interest to know how closely the embryo may be invested with tissues bearing the infectious principle of the mosaic disease. Before the question had been fully investigated the writer was under the impression that the virus ordinarily did not reach the placental column bearing the seeds. In order to test this point, three healthy Connecticut Broadleaf tobacco plants were set aside until seed production had begun. The spongy placental tissue of six to eight capsules on each plant was then punctured deeply with a needle and the virus of mosaic disease introduced abundantly. Capsules of all ages, from very young to those fully grown, were punctured and the virus injected. Although a number of the more immature capsules developed very poorly following this treatment, an abundance of seed was secured and sowed on March 31, 1914. From this seed 400 plants were obtained and later transplanted to 3-inch pots. On May 18 all were healthy, and 40 were inoculated with the virus of the mosaic disease. Practically all of those inoculated were showing symptoms of the disease on May 27 and 28.

Later experiments with affected plants have shown that the capsules of such plants normally contain the virus of the disease. The tobacco capsule contains two cells formed by a median cross wall or partition. By cutting through the thin ovary wall near this partition on both sides of the capsule the ovary wall can be readily removed in two halves, exposing to view each half of the large placental column with its attached

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ovules. A thin, sharp scalpel heated to redness was used for cutting away the ovary wall, so that possible infection of any portion of the placental tissues from the ovary wall itself was avoided. Table I shows the occurrence of virus in the placental structure and ovules of mosaic-diseased plants.

TABLE I.—*Occurrence of virus in the placental structure and ovules of tobacco plants affected with the mosaic disease*

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914. Apr. 23	10	Connecticut Broadleaf.	Sap of portions of placental column and immature ovules of green capsules from plants affected with mosaic disease. These portions were macerated in a mortar with clean tap water.	8 affected with mosaic disease on May 9.
23	10	do	Sap of green leaves from same plants.	6 affected with mosaic disease on May 9.
23	10 (control)	do	Sap of green placentas and ovules from a healthy plant and macerated with tap water.	All healthy on May 9.
May 18	10	Maryland Mammoth	Sap of macerated placentas and immature ovules of large, green capsules of plants affected with mosaic disease.	6 affected with mosaic disease on May 26.
18	10	do	Sap of ovaries entire from the same plants.	10 affected with mosaic disease on May 26–28.
18	10	do	Thin paste obtained by grinding in a mortar with a small quantity of tap water the white and brownish immature seeds of two capsules from plants affected with mosaic disease. These seeds were scraped very carefully from the placental column.	4 affected with mosaic disease on May 28.
18	10	do	Sap of two placentas alone, from which the ovules were removed in the preceding test.	7 affected with mosaic disease on May 28.
18	10 (control)	do	Sap of immature seeds and placentas obtained from a healthy plant and ground with tap water.	All healthy on May 28.
28	10	do	Macerated placentas and immature seeds of green capsules from plant A, affected with mosaic disease.	10 affected with mosaic disease on June 6.
28	10	do	Thoroughly mature, loose seeds from dried, brown, matured capsules of the same plant, A, were poured from the capsules into a mortar and ground to a thin paste with tap water.	3 affected with mosaic disease on June 8.
28	10 (control)	do	Macerated placentas and immature seeds of green capsules from a healthy plant, mixed and ground in a mortar with dried mature seeds from the same plant. A small quantity of tap water was added to obtain a thin paste.	All healthy on June 8.
June 2	10	do	Macerated white immature ovules carefully removed from the spongy, succulent placentas of green capsules of plants affected with mosaic disease and mixed with tap water to form a thin paste.	4 affected with mosaic disease on June 10.
2	10	do	Sap of leaves from the same plants affected with mosaic disease used in the preceding test.	10 affected with mosaic disease on June 10.

TABLE I.—*Occurrence of virus in the placental structure and ovules of tobacco plants affected with the mosaic disease*—Continued

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914.				
June 2	10.....	Maryland Mammoth..	Thin paste obtained by grinding with tap water in a mortar thoroughly dry, loose, ripened seeds from matured capsules of plants affected with mosaic disease.	4 affected with mosaic disease on June 10.
2	10.....	.....do.....	Same macerated material used as in preceding test.	2 affected with mosaic disease on June 10.
2	10 (control)..	.....do.....	Paste obtained by grinding together white immature ovules from green capsules and dry, loose, ripe seeds from healthy plants. Small quantity of tap water added to thin the paste.	All healthy on June 10.
June 4	10.....	.....do.....	Thin paste obtained by grinding with tap water loose, dry, thoroughly ripened seeds of capsules from plants affected with mosaic disease.	7 affected with mosaic disease on June 10.
4	10.....	.....do.....	Sap of green leaves from the plants in the preceding test.	10 affected with mosaic disease on June 10.
4	10.....	.....do.....	Paste obtained by grinding and thinning with tap water dry, loose, ripe seeds from capsules of plant B affected with mosaic disease.	1 affected with mosaic disease on June 10.
4	10.....	.....do.....	Thin paste obtained by grinding with tap water the nearly mature, light brown seeds from ripening capsules of the same plant B affected with mosaic disease. In this test the capsules selected were still green and the placental column succulent and full. The seeds, which were firm and brownish in color, still adhered to the surface of the placenta.	3 affected with mosaic disease on June 10.
4	10 (control)..	.....do.....	Paste obtained by macerating in a mortar with tap water dry, loose seeds, nearly matured seeds, and leaves of healthy plants.	All healthy on June 10.
5	10.....	.....do.....	Paste obtained by macerating with tap water the loose, dry seeds from ripening capsules of plants affected with mosaic disease. These seeds were mature, but the placental column was still succulent, although beginning to dry and shrink somewhat.	2 affected with mosaic disease on June 11.
5	10.....	.....do.....	Macerated placentas from which the seed in the preceding test was removed. Small quantity of tap water added to obtain a thin paste.	8 affected with mosaic disease on June 11 and 12.
5	10 (control)..	.....do.....	Paste obtained by macerating with tap water in a mortar the dry, loose seeds and placentas of capsules obtained from healthy plants.	All healthy on June 11 and 12.

Earlier experiments[1] have shown that the roots, the apparently healthy lower leaves, and the corollas of plants affected with the mosaic disease sooner or later carry the virus of the disease. More recently experiments have been carried out to determine whether the virus is present

[1] Allard, H. A. Mosaic disease of tobacco. U. S. Dept. Agr. Bul. 40, p. 18–19. 1914.

in the filaments, anthers, and pistils of blossoms produced by affected plants. See Table II.

TABLE II.—*Occurrence of virus in the filaments, anthers, and pistils of blossoms produced by tobacco plants affected with the mosaic disease*

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914. May 21	10	Maryland Mammoth	Sap of macerated pistils extracted very carefully with forceps from the blossoms of a tobacco plant affected with mosaic disease. A gentle pull with the forceps readily severs the style at its junction with the apex of the ovary.	10 affected with mosaic disease on May 28.
21	10	do	Sap of leaves of the same plant, A.	Do.
21	10 (control)	do	Sap of the leaves and pistils of a healthy plant.	All healthy on May 28.

Experiments with the pistils of plants affected with the mosaic disease were again repeated, using only the upper portion of the style and the stigma. This was done to avoid the possibility of infection from tissues of the ovary adhering to the base of the style when extracted. See Table III.

TABLE III.—*Occurrence of virus in the upper portions of the filaments, anthers, and pistils of blossoms produced by tobacco plants affected with the mosaic disease*

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914 May 27	10	Maryland Mammoth	Macerated upper portions of pistils from plants affected with mosaic disease.	8 affected with mosaic disease on June 6.
27	10	do	Sap of leaves of the same plants.	10 affected with mosaic disease on June 6.
27	10 (control)	do	Sap of leaves and upper portions of the pistils of healthy plants.	All healthy on June 6.
June 2	10	do	Sap of anthers of plants affected with mosaic disease. These anthers were carefully removed with forceps just prior to opening, and were macerated in a mortar with a small quantity of clean tap water sufficient to make a thin paste.	10 affected with mosaic disease on June 10.
2	10 (control)	do	Sap of anthers of healthy plants extracted in the same manner.	All healthy on June 10.

From the preceding experimental data it is evident that the virus of the mosaic disease in affected plants becomes distributed throughout the placental structures, reaching even the ovules themselves. Whether the virus passes beyond the integuments of the ovules to the embryo sac has not been determined. There is some indication that the macerated placenta in a succulent condition is more effective than the immature

ovules, and especially the loose, dry, normally ripened seeds, in producing the mosaic disease in inoculated plants. Although the greatest care may be exercised in removing immature seeds from a succulent placental column, it must be evident that the probability of rupturing and removing some of the placental substance is very great. In the normal ripening process, however, the seeds loosen and fall away from the drying and shrinking placental column so gradually that the minimum amount of placental material is carried away attached to the seeds.

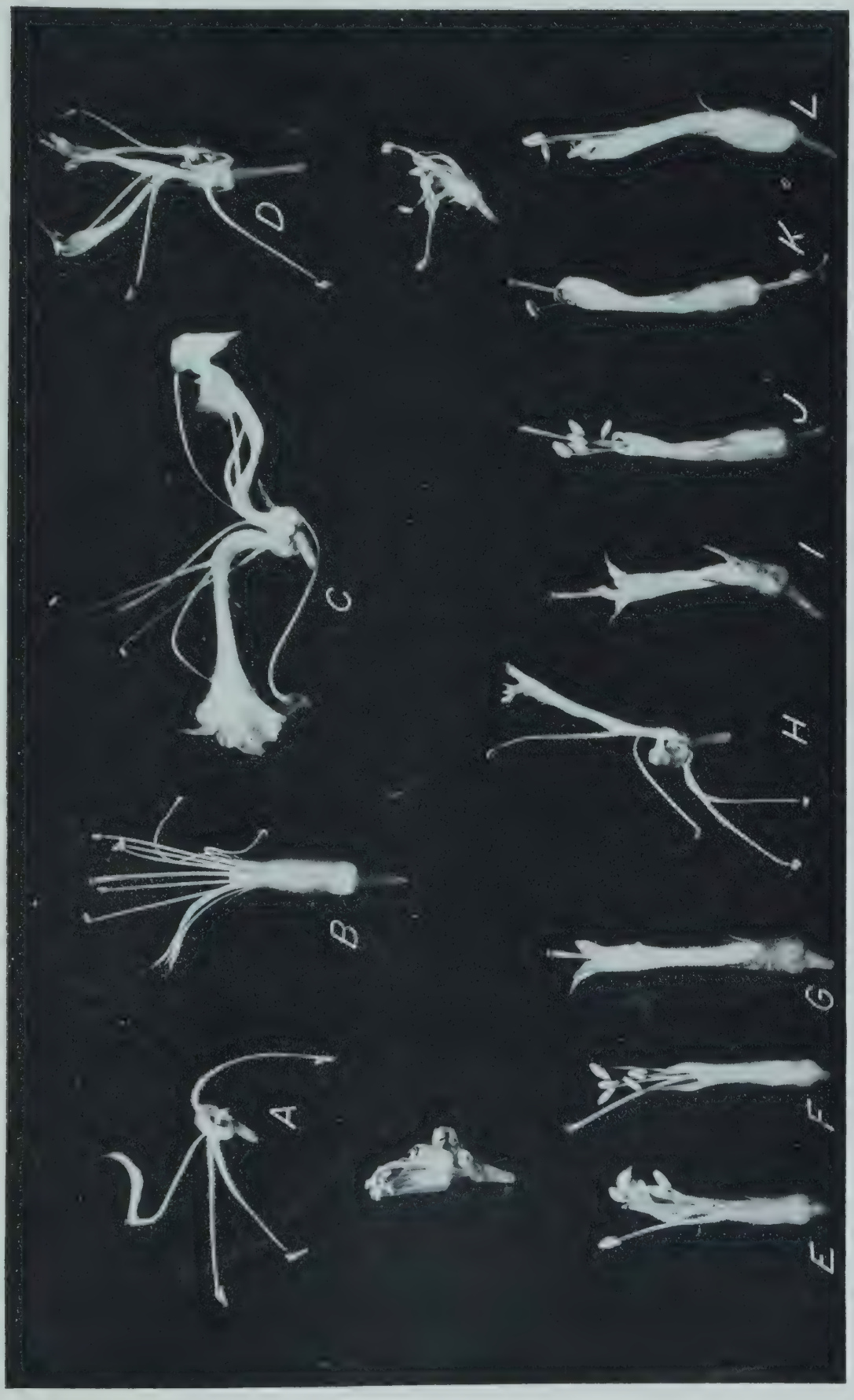
Malformations caused by the mosaic disease may disturb the normal relations of stamens and pistils to such an extent as to cause sterility in many blossoms, owing to the failure of natural self-pollination. Hand pollination of these pistils has frequently led to normal seed development. Not infrequently the development of the corolla is almost entirely inhibited and the stamens and pistils also fail to develop normally. Even in these blossoms the anthers may contain more or less functioning pollen, which has produced normal fertilization when transferred to the pistils of healthy blossoms. In some instances the anthers produce little or no functioning pollen. In extreme cases the normal form and structure of the anther sacs is replaced by a mass of irregular proliferations. Generally blossoms affected with the mosaic disease appear to produce viable pollen and ovules quite as freely as those borne by healthy plants (Pl. XXIII).

From the fact that the mosaic disease is not known to occur as the result of embryonic transmission of the disease directly from the mother plant during seed development, it is evident that a very efficient barrier guards against embryonic infection or the subsequent successful continuation of the disease from parent to seedling. In particularly malignant cases of the disease, where few or no viable seed are produced, following pollination with pollen from healthy blossoms, it is possible that the infective agents of the disease have produced embryonic infection which resulted in death. Whether the failure to produce viable seed in these instances is due to actual infection of the ovules or to a general impairment of nutrition and cell division of the capsular structures associated with embryonic development, can not at present be determined. It is possible that embryonic development never proceeds in those ovules actually invaded and infected by the virus of the disease. In all experimental tests at least germinable seeds from plants affected with the disease have always produced normal, healthy offspring.

At this time speculation seems quite fruitless, and one can only wonder what protects the embryo so securely from the mosaic disease, even though intimately associated with and nourished by infective parental tissues.

PLATE XXIII

Malformed blossoms of tobacco (*Nicotiana tabacum*) caused by the mosaic disease, which is often responsible for the various abnormalities shown. The corolla may show mottling only, or it may develop very imperfectly, producing various degrees of catacorolla, fasciation, etc. In some instances the corolla fails to develop entirely. The plants producing these acquired abnormalities as a result of the mosaic disease have been studied as to their inheritance, but the descendants were healthy and their blossoms normal. A common cause of sterility is the failure of successful pollination of the stigma, owing to the abnormal displacement of pistil and stamens. Hand pollination of such blossoms has often given capsules containing an abundance of fertile seed. Blossoms as poorly developed as A, D, and H are usually incapable of producing seed. The anthers, however, sometimes contain functioning pollen which may produce fertilization of the ovules when transferred to the pistils of healthy blossoms. Blossoms E, F, G, I, J, K, and L usually produce seed if hand pollination is practiced.


A
B
C
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E
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K
L

DISSEMINATION OF BACTERIAL WILT OF CUCURBITS

[PRELIMINARY NOTE]

By FREDERICK V. RAND,

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In the discussion of his exhaustive studies upon bacterial wilt of cucurbits, Dr. Erwin F. Smith [1] makes the following statements relative to certain still unsolved portions of the wilt problem:

> Leaf-eating insects, and especially *Diabrotica vittata* (fig. 55), are, I believe, the chief agents in the spread of this disease. They feed readily, and sometimes the writer has thought preferably (fig. 7), on wilted leaves which are swarming with this organism. In this way their mouth-parts can not fail to become contaminated and to serve as carriers of the sticky infection. No other means of dissemination is known to the writer, and this is believed to be the common way in which the disease is distributed. * * *
>
> Seasonally the disease does not manifest itself until the leaf-eating beetles have put in their appearance, and this has led to the suspicion that the organism might pass the winter inside the bodies of these hibernating insects (*Diabrotica vittata*). As to this nothing definite is known.

He has referred to this subject again in his St. Louis address,[2] as follows:

> The writer has since proved several diseases to be transmitted by insects, notably the wilt of cucurbits, and here the transmission is not purely accidental, but there appears to be an adaptation, the striped cucumber beetle (*Diabrotica vittata*), chiefly responsible for the spread of the disease, being fonder of the diseased parts of the plant than of the healthy parts. This acquired taste, for it must be that, works great harm to melons, squashes, and cucumbers. Whether the organism winters over in the beetles, as I suspect, remains to be determined. Certainly the disease appears in bitten places on the leaves very soon after the spring advent of the beetles.

It was especially with a view toward throwing some light on the mode of hibernation of the causal bacteria and of developing some practical method of control that the writer undertook to continue the studies upon this frequently very destructive disease. Since the study was begun in midsummer (July, 1914), the first season's work consisted largely of field observations which covered the territory from eastern Long Island, N. Y., and Maryland to Indiana and Wisconsin. Some of the worst examples of injury from wilt were found in eastern Long Island, and accordingly this locality was selected for the field tests of the following season (1915). While further investigations are under way, it appears

[1] Smith, Erwin F. Bacteria in Relation to Plant Diseases. v. 2, p. 215. Washington, D. C., 1911.

[2] ———. A conspectus of bacterial diseases of plants. *In* Ann. Mo. Bot. Gard., v. 2, no. 1/2, p. 390, 1915.

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desirable to record at this time the result of the first season's experimentation.

At East Marion, Long Island, N. Y., two fields were selected where during the season of 1914 about 75 per cent of the cucumber vines (*Cucumis sativus*) had been destroyed by bacterial wilt, as determined by the writer. Here was an excellent environment in which to test the question as to hibernation of the bacteria in soil *v.* animal carriers. Fifty large frame cages 4 feet square and 3½ feet high were constructed. The lower 18 inches of the sides were boarded up, while the covers and the upper 2 feet were inclosed in 18-mesh wire mosquito netting. These bottomless cages were set 15 inches into the soil, leaving 3 inches of the boarded portion above the soil line. The juncture between cover and sides was sealed with cotton and liquid tar, and the cracks between the boards of the basal portion were stuffed with cotton to prevent access of insects. Twenty-three of these cages were set in one of the fields and twenty-seven in the other. In each field the soil in four cages was sterilized by live steam at 75 pounds' pressure for one hour, but this made no difference in the final result. This was done in order to kill any wilt bacteria which might have wintered over in the soil. In each field the cages containing sterilized and unsterilized soil were located at intervals across the field and cucumbers were planted in the usual way in the soil between and within the cages on June 5 and 6. A half-dozen plants were grown in each cage and later on thinned to three or four. After planting, the cages were all sealed with lead seals to preclude accidental opening of the covers, and whenever necessary to open the cages for examination they were again sealed in the same manner. By this careful construction and setting of the cages it was thought possible to exclude all of the insects injurious to cucumbers except possibly aphides and flea beetles, some of both of which later on entered some of the cages through the wire netting, but were without effect upon the experiment.

Field No. 1 was separated by at least one-half mile, including a quarter-mile depth of woods, from the nearest cultivated cucurbits. It was, in fact, surrounded on three sides by woods and on the fourth side by Long Island Sound.

Field No. 2 was about one-quarter mile from other cucurbits, but without the intervening woodland.

Plate XXIV, figure 1, shows the cages in place in field No. 2; Plate XXIV, figure 2, shows field No. 1, with a cage in the foreground lifted, the darker part of the base indicating the depth buried.

No commercial cucumber fields were planted in either locality until two or more weeks later in the season.

As soon as the young plants were 2 or 3 inches high and before any wilt had appeared, five or six striped cucumber beetles were introduced into each of 4 cages, 2 in each experimental field. These beetles were

collected in field No. 1, where presumably they had hibernated. Within a week several plants in 1 of the 2 cages in field No. 1 into which the beetles had been introduced showed signs of wilt, starting from points in the leaves gnawed by the beetles. Upon cutting off the stems the typical stringing out of the viscid white bacterial slime was seen. Cultures were made by the writer from one of these plants and subsequent inoculations from these cultures into healthy plants again gave the disease. Other wilted plants from the same cage were sent to Washington, D. C., and from one of these *Bacillus tracheiphilus* was obtained and with it successful inoculations were made in cucumbers in one of the Department greenhouses by Dr. Smith. No signs of wilt occurred in the 3 other cages in which beetles were placed, or, with one exception, in any of the 46 other cages.

Meanwhile in both fields the wilt was beginning on plants between the cages. At first the wilt appeared only on a plant here and there, and then gradually extended throughout the two fields until no portion was entirely exempt. In the two fields together there were in the neighborhood of 1,200 hills of cucumbers exposed to attack of the beetles. The cages in field No. 1 extended approximately a quarter of a mile through the field at equal distances and in field No. 2, which was about two-thirds as large, they were spaced closer. There was a check plot contiguous to each cage. The approximate number of cases on the plants in field No. 1 during the three months was 600; in field No. 2 it was 200. No counts were made after September 1, owing to the appearance of the cucumber mildew (*Plasmopora cubensis*).

In all these cases of wilt in the exposed (uncovered) plants, infection was clearly seen to have started from beetle injury. Careful record was kept throughout the season of every hill and plant showing wilt, and although between the cages the disease was everywhere present the plants within the cages were strikingly free from the disease. The plants in these 50 cages were examined every day from planting time (June 5–6) until September 1. In one cage where beetles were not liberated, wilt was noted just starting in the tip leaf of one plant at a point gnawed by a beetle. A careful search in this cage disclosed a striped beetle, which was summarily disposed of. Microscopical examination and cultures from the lower part of the stem failed to disclose any bacteria, showing that the wilt in this case could not have come from the soil and must have been brought in by the beetle, which probably entered through a crack due to warping of the boards. Careful search failed to disclose any further beetle injury within the cage, and, after the removal of the beetle and the one wilting plant, no further signs of the disease appeared therein during the season. With this exception and that of the above-mentioned 1 cage into which the beetles were purposely introduced, not a case of wilt occurred in any of the 50 cages during the entire season.

From these cage experiments therefore it would appear that the wilt bacteria are carried over the winter by the hibernating beetles and inoculated into the cucumbers as they feed upon the young leaves. However, from the fact that wilt appeared in only one of the four cages into which beetles were introduced, it would seem that not all hibernating beetles carry the disease, but only those, or some of those, which have previously fed upon wilted plants. In other words, the beetles act not only as summer but also as winter carriers of the wilt organism from one cucumber plant to another. At least the above facts seem to warrant this as a tentative conclusion. The only possible alternative is to suppose that some of the beetles captured on June 17 and introduced into the four cages had recently had opportunity to gnaw diseased plants, which under the circumstances of their capture appears to the writer out of the question. Finally, in addition to the positive evidence of insect transmission afforded by this cage and by the one into which a beetle accidentally penetrated, as well as by daily observation on the check plants, there is the negative evidence afforded by the fact that in all cages from which beetles were excluded the plants remained free from the disease in two fields where it was very prevalent.

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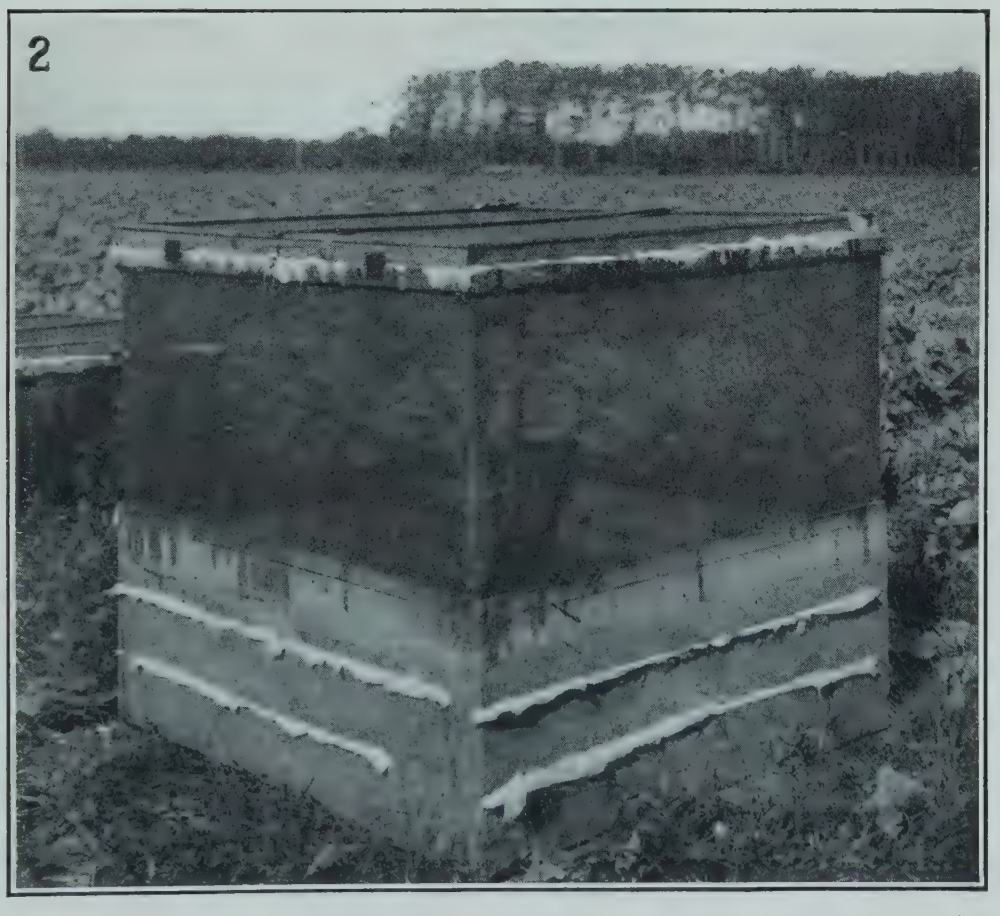
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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., NOVEMBER 15, 1915 NO. 7

GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL.[1]

By W. A. WITHERS and F. E. CARRUTH,[2]
North Carolina Agricultural Experiment Station

TOXICITY OF COTTONSEED

The term "cottonseed meal" is applied to the ground cake left after the oil is pressed from the seed of cotton (*Gossypium* spp.). For many years it was regarded as a by-product of little value. It is now used extensively as a feed. The annual production of the United States is about 2,000,000 tons, valued at about $53,000,000. While it may be fed profitably to horses, cattle, sheep, etc., in moderate amounts, poisoning and often death occur as a result, especially if the animal has not been gradually accustomed to it. It is generally avoided as a feed for pigs on account of the numerous deaths associated with its use. Dinwiddie (1905) states that hogs show no greater susceptibility than cattle when fed quantities proportional to their body weight. Feeding experiments at the North Carolina Experiment Station have shown that where swine are fed one part of cottonseed meal with three parts of corn meal death generally ensues in from five to seven weeks, although some pigs have been fed for a year or more without fatal results.

In a recent experiment at this Station nine pigs weighing from 75 to 150 pounds were fed in a closed pen on a daily ration of 1 per cent of cottonseed meal and 3 per cent of corn meal, based on their initial body weight. Six died between the thirty-fifth and the fifty-seventh day. The others were alive on the ninetieth day. Roughly, then, 45 per cent of their initial weight in cottonseed meal was fatal to these pigs. All the smaller pigs died.

Withers and Brewster (1913) found that rabbits and guinea pigs would succumb in about 13 days (6 to 22 days) when fed at the rate of 1 per cent of initial body weight daily. Experiments with 22 rabbits showed that, on

[1] This paper is the third in a series of "Studies in Cottonseed Meal Toxicity." Study I, Withers and Ray (1913), is a criticism of Crawford's pyrophosphoric-acid hypothesis; Study II, Withers and Brewster (1913), suggests iron salts as an antidote.

[2] For their cooperation with us in this investigation, we desire to thank Dr. G. A. Roberts and Dr. W. B. Smith, of the Veterinary Department, and Dr. B. F. Kaupp, Pathologist, of the Poultry Department, North Carolina Experiment Station.

an average, 8.3 per cent of initial body weight was sufficient to cause death. These authors make the following statement in regard to these tests:

> As a rule the rabbits ate the meal well during the first few days and made gains in weight. But towards the end they began to refuse the meal in whole or in part and soon thereafter died.

There have been numerous suggestions as to the cause of poisoning and death from the feeding of cottonseed meal. These are summarized in the Experiment Station Record (1910, p. 501) as follows:

> It has been variously ascribed to the lint, the oil, the high protein content, to a toxalbumin or toxic alkaloid, to cholin and betain, to resin present in the meal, and to decomposition products.

Pathogenic organisms and certain fungi have also been suggested.

Friemann (1909), a veterinarian, obtained from the alcoholic extract of cottonseed meal which had caused sickness in cattle a base the platinum salt of which contained 28.75 per cent of platinum. The free base had a paralytic action on exposed frogs' hearts similar to muscarin. He concluded that the toxicity was to be referred to ptomains which result from the nitrogen-containing components of the lecithin, and that unsaturated fatty acids probably contributed to the total action of the meal.

Crawford (1910) concluded that "the chief poisonous principle in certain cottonseed meals is a salt of pyrophosphoric acid." This conclusion is discussed later in this article.

Withers and Ray (1913b) found that the toxicity of cottonseed meal could be destroyed by boiling it with alcoholic caustic soda. This was the only solvent of a large number used which removed or appreciably affected the toxic principle. A noteworthy fact is that the neutralized and evaporated extract was shown to be nontoxic.

Withers and Brewster (1913) found that if a solution of iron and ammonium citrate was fed with cottonseed meal rabbits did not die during a period about seven times as long as the feeding period when iron salts were omitted. Furthermore, rabbits made sick on the meal recovered when the iron solution was supplied with the meal.

PREPARATION OF GOSSYPOL

Our recent experiments have led us to believe that gossypol is the toxic substance of cottonseed.

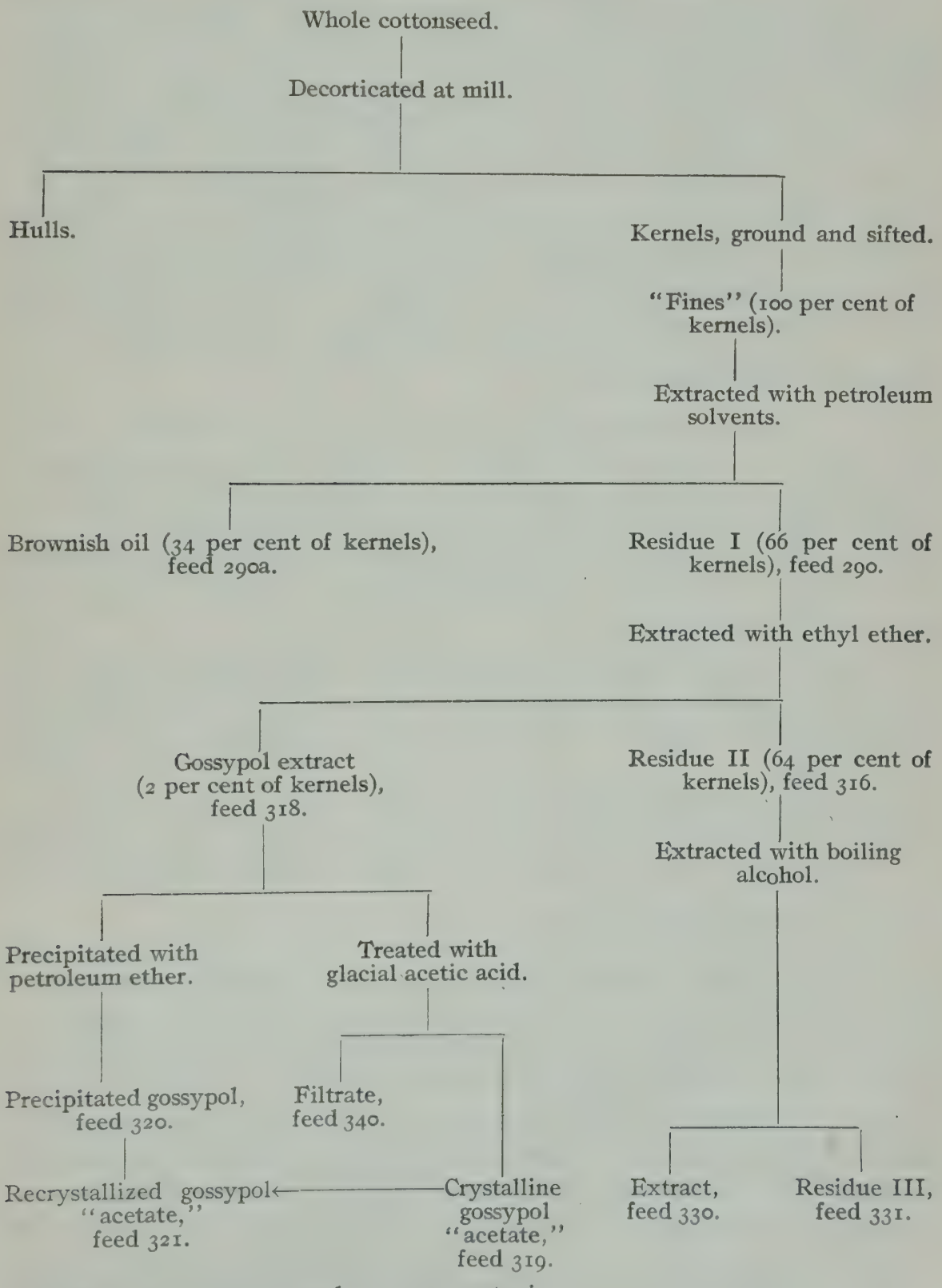
In our previous experiments we used cottonseed meal as the material for study, but in the experiments discussed in this paper we used cottonseed kernels as the initial substance, as gossypol is more readily and more completely extracted from the kernels than from the meal. Generally speaking, the meal and the kernels are toxic to rabbits to the same degree.

We extracted gossypol from ground cottonseed kernels with ethyl ether, after previously removing most of the oil with petroleum ether or gasoline. Gossypol was separated from the ethereal solution by evaporation, by precipitation with petroleum ether, or by precipitation with acetic acid.

These products, differing in purity, have been designated by us as "gossypol extract," "precipitated gossypol," and "gossypol 'acetate.'" All proved toxic to rabbits.

The method of preparing the gossypol and other feeds is shown in the accompanying outline.

OUTLINE OF METHOD OF PREPARING FEEDS


Whole cottonseed.
Decorticated at mill.
Hulls.
Kernels, ground and sifted.
"Fines" (100 per cent of kernels).
Extracted with petroleum solvents.
Brownish oil (34 per cent of kernels), feed 290a.
Residue I (66 per cent of kernels), feed 290.
Extracted with ethyl ether.
Gossypol extract (2 per cent of kernels), feed 318.
Residue II (64 per cent of kernels), feed 316.
Extracted with boiling alcohol.
Precipitated with petroleum ether.
Treated with glacial acetic acid.
Precipitated gossypol, feed 320.
Filtrate, feed 340.
Recrystallized gossypol "acetate," feed 321.
Crystalline gossypol "acetate," feed 319.
Extract, feed 330.
Residue III, feed 331.

Feeds 290, 318, 319, 320, and 321 are very toxic.
Feed 316 is very slightly toxic after long feeding.
Feeds 331 and 340 are nontoxic.

OCCURRENCE AND PROPERTIES OF GOSSYPOL

If the cottonseed kernel is examined with a lens, many small yellowish brown to black spots may be seen (Pl. XXV). They are referred to by Hánausek (1907, p. 367) as "secretion cavities" in the following statement:

> Distributed among the mesophyll cells [of the cotyledons] are procambium bundles and globular, lysigenous **secretion cavities** (*se*) 100—400μ diameter. The lysigenous character of these cavities when mature is quite clearly evident. The tissue which surrounds them consists, in its outer portion, of tangentially flattened, very thin-walled cells, and within the last a mucilaginized layer in which the traces of the cell walls are still evident. This colorless mucilage layer, which treatment with hydrochloric acid and, after washing with water, with potash brings out as a yellow, folded, and laminated mass, encloses the greenish-black, opaque secretion (*v*). Since the mucilage layer is soluble in water, the secretion flows out from the sections laid in water in the form of a thick emulsion consisting of a colorless mass containing minute dark-colored grains (resin?) in lively molecular motion. Chlorzinc iodine colors the secretion red-brown, sulphuric acid dissolves it to a thick turbid fluid of a blood-red color. Ammonia colors the liquid greenish yellow without destroying the emulsion. Potash also imparts a green color.

They are designated by Watt (1907, p. 56) as "gland dots" and by Balls (1912, p. 13) as "resin glands." From these glands we have extracted gossypol and for clearness have alluded to them as gossypol glands. Their function does not seem to be very well known.

They occur in all parts of the cotton plant and in all varieties which we have seen. They are very abundant in the cambium layer of the bark of the cotton root.[1]

Gossypol was first isolated by Marchlewski (1899) from the "foots" in the purification of cottonseed oil, and on account of its source and phenolic properties he proposed for it the name "gossypol," from Gossyp [ium phen]ol.

Previous to Marchlewski's work the crude substance constituting the coloring matter of cottonseed oil was referred to by the older writers—e. g., Hanausek (1903, p. 755)—as "gossypin,"[2] which is described as a light-brown pungent powder.

Marchlewski (1899) proposed for gossypol the formula $C_{13}H_{14}O_4$, with $C_{32}H_{34}O_{10}$ as an alternate formula. Among its properties as described by him are the following: A beautifully crystalline yellow-colored dihydroxy phenolic substance, easily soluble in alcohol, benzene, chloroform, ether, acetone, and glacial acetic acid; insoluble in water; soluble in concentrated sulphuric acid with a magnificent red color; easily soluble in alkalies, the solution for the first second being yellow, after a short time becoming a beautiful violet and then fading, the changes being due to oxidation. The alcoholic solution gives a dark-green color with ferric

[1] Thus, we have an indication that gossypol may be the active principle of the medicinal extract of cotton-root bark. (Bouchelle, 1840.)

[2] The original work on gossypin has not been located by us.

chlorid. The samples dried at 125° to 130°, melted at 179° to 180°, and air-dried samples melted with quick heating at 188°.

Our experiments indicate that the substance which Marchlewski named "gossypol" contained acetic acid in combination with the substance to which we think the name "gossypol" should be assigned. The acetic-acid content of our different products varied from 8.5 to 9.5 per cent, depending upon the conditions under which crystallization took place. The substance containing acetic acid and the substance freed of acetic acid differ in elementary composition and in melting point, as one would expect. Marchlewski's empirical formulæ for gossypol appear to us to be erroneous, as they were based upon the ultimate analysis of the acetate instead of the substance freed from acetic acid.

Marchlewski supposed that gossypol might prove of value as a dyestuff, and before the publication of his article took out patents [1] to protect his discoveries. He made no suggestion as to its physiological activity, nor have we been able to find that anyone else has done so.

EXPERIMENTAL WORK WITH GOSSYPOL

METHOD OF ROUTINE FEEDING

Rabbits and guinea pigs were used in our experiments. Rabbits do not eat cottonseed meal nor cottonseed kernels readily. Therefore, to make the various solid feeds palatable, we moistened them with the best grade of molasses, rabbits eating the various feeds with great relish until made sick. They were fed liberally with green feed once a day.

In case of forced feeding a catheter was inserted to the stomach and the dose allowed to drain in. The intraperitoneal injections were made by the Station veterinarian, Dr. G. A. Roberts, by whom also the post-mortem examinations and notes were made.

The rabbits were fed in galvanized-iron cages, about 20 inches long by 16 inches wide by 10 inches deep. Each contained a trough with separate compartments for water and feed.

TOXICITY OF COTTONSEED KERNELS (FEED 290)

Cottonseed kernels were extracted with petroleum ether, which does not remove gossypol in appreciable quantities. A rabbit was started on 15 gm. daily of this feed, but it would not eat all of it. Diarrhea resulted on the second day, and its appetite for green feed was affected on the third and fourth days. It gradually ate less and less, so that the feed was discontinued on the eleventh day and the ether-extracted kernels (feed 316) substituted on the day following. During the last five days it ate only 11.5 gm. It ate 56.5 gm. of feed 290, losing 130 gm. in weight, but recovered on feed 316.

[1] English patent No. 24418 of 1895 and German patents Nos. 98074 and 98587 of 1898.

Two guinea pigs, A and B, were tried with this feed. Guinea pig A was off its feed at the time from eating precipitated gossypol spread on corn meal (feed 318). An attempt was made to give it kernels in which the gossypol was not so easily detected, but the animal would not touch them.

Guinea pig B had eaten feed 316 for 50 days and had gained in weight. After it had been on corn meal and molasses (feed 317) for about a week, it was placed upon feed 290 (7 gm. of kernels with molasses). It ate only 4 gm. of the kernels, although other feed was withheld for a day. We concluded from this that even the 4 gm. had affected it physiologically and had made it suspicious of the feed. After a week upon control feed, it ate feed 316 without objection.

Rabbit 957, which had eaten feed 316 for 46 days without noticeable effect, was rested for three weeks and then fed the residue after petroleum-ether extraction, which does not remove the gossypol. Its appetite was perceptibly affected on the third day, but it ate most of the feed for 6 days. On the ninth day it refused to eat feed 290, but ate green feed slowly. It died on the fourteenth day, showing symptoms of cottonseed-meal poisoning. See Table I.

TABLE I.—*Results of feeding cottonseed kernels (fat-free; feed 290) and cottonseed meal to rabbits and guinea pigs*

Feed and animal No.	Weight of animal.			Weight of feed eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Cottonseed kernels:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 958.....	1,560	1,430	130	56.5	85	11	Made sick and refused to eat.
Guinea pig A.....	680	.....	.....	0	0	1	Refused feed entirely.
Guinea pig B.....	650	.....	.....	4	6	1	Refused the feed.
Rabbit 957.....	1,800	1,535	235	100	150	14	Died.
Cottonseed meal:[a]							
Average for 22 rabbits.	1,577	1,238	339	[b] 133	.....	13	All died.

[a] The results of Withers and Brewster's experiments (1913) with cottonseed meal are here inserted for comparison.

[b] Each rabbit consumed from 48 to 225 gm. of cottonseed meal and died upon the feed in from 6 to 22 days.

TOXICITY OF GOSSYPOL EXTRACT

It is much simpler to prepare gossypol from cottonseed than from the oil.[1] Qualitative tests of ground cottonseed showed that gossypol could be extracted with ether, carbon bisulphid, chloroform, benzene, alcohol, but not with petroleum ether or gasoline. By extracting the

[1] This point will be discussed under the chemistry of gossypol, which will appear in a subsequent publication.

ground kernels in a Soxhlet apparatus for several hours with petroleum ether and then with ethyl ether we obtained a product which for convenience we called "gossypol extract." After the evaporation of the ether there was left a red resinous material which had a peculiar pungent odor and which amounted to about 2.5 per cent of the weight of the kernels used. This material seems to consist largely of gossypol, although we have not yet made an examination with reference to identifying other constituents. No doubt considerable oil is present.

Gossypol extract administered intraperitoneally and fed in one large dose in oil or in small daily doses with corn meal and molasses was found to be toxic to all the animals experimented with.

Catheter Feeding of Gossypol Extract

This gossypol extract from 90 to 180 gm. of cottonseed kernels was fed to each of four rabbits and proved fatal in every case. Care was taken to remove all the solvent, and the gossypol extract was dissolved in cottonseed oil which had been purified in this laboratory. The oil solution was then fed through a catheter. The control animal, on a large dose of cottonseed oil, had diarrhea the next day, but was normal thereafter. Table II summarizes the results obtained with the gossypol extract fed forcibly to rabbits.

Table II.—*Results of feeding gossypol extract and purified cottonseed oil with a catheter to rabbits*

Feed and rabbit No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	*Gm.*	*Gm.*	*C. c.*	
923	1,500	90	15	Died in about 12 hours.
924	1,750	180	30 (½ water.)	Died in 30 to 40 hours.
926	3,000	About 160	30–35	Died in 25 hours.
927	2,100	170	30 (⅔ water.)	Died in less than 16 hours.
Purified cottonseed oil:				
925 (control)	2,500	.....	30–35	Sick with diarrhea next day only.

POST-MORTEM OBSERVATIONS

Rabbit 923.—Part of dose still in stomach. First foot of intestines considerably injected. Excess serous fluid in abdomen, 10 c. c. No evidence of catheter reaching lungs.

Rabbit 924.—Lungs very much congested. Excess fluid in chest cavity, 3 to 4 c. c. Some hemorrhagic condition along blood vessels of large intestines.

Rabbit 926.—Lungs normal. Anus discolored from diarrhea.

Rabbit 927.—Lungs markedly congested.

Intraperitoneal Injection of Gossypol Extract

Cottonseed oil was used as the vehicle for the injection of the gossypol extract. This was readily available and of suitable consistency for injection. It was purified in this laboratory from a sample of crude oil. This oil was selected chiefly for its ability to hold the gossypol extract in solution. Crawford (1910, p. 531–532), under "Experiments with cottonseed oil," makes the following observations:.

> After feeding a large dose of the crude cottonseed oil (25 c. c.) to a rabbit its weight steadily fell and remained low, and when a moderate dose (15 c. c.) was fed and this was followed by repeated small ones the animal died, showing irritation of the gastro-intestinal canal. Lendrich [1908] noted that after the daily administration of cottonseed oil his rabbits emaciated, but readily assimilated the same dose of oil that was given intraperitoneally.
>
> After feedings with purified cottonseed oil, or with olive oil, there was a loss in weight, but the animals did not die. After feeding pure cod-liver oil the animals died. The loss in weight was small in the case of feeding purified cottonseed oil. The fact that the cottonseed oil gave no red reaction to litmus paper would suggest that the loss in weight, noted after feeding the crude oil, was not due to the free oleic acid. This acid has recently been shown to play an important rôle in the production of certain forms of anemia. Oils interfere with gastric digestion in man, and this fact must be taken into consideration in experiments on such animals as rabbits.

Two controls receiving purified cottonseed oil were affected to only a slight extent. All five rabbits receiving intraperitoneally an oil solution of gossypol extract died, the extract being the equivalent of from 45 to 85 gm. of cottonseed kernels. See Table III.

Table III.—*Results of intraperitoneal injection in rabbits of gossypol extract dissolved in purified cottonseed oil*

Feed and rabbit No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	*Gm.*	*Gm.*	*C. c.*	
931	770	About 50	8	Died.
932	600	About 45	15 (½ water.)	Do.
934	937	85	5–6	Do.
928	1,090	85	7	Do.
929	1,225	About 50	4	Do.
Purified cottonseed oil:				
930 (control)	864	.....	10	Only slightly indisposed.
933 (control)	864	.....	10	Do.

POST-MORTEM OBSERVATIONS

Rabbits 931 and 932.—Fatal with complications in four days. Entire belly (subcutaneous) very edematous. Part of dose was injected subcutaneously.

Rabbit 934.—Died between the seventh and the nineteenth hour. Considerable serous fluid in abdomen. Serous fluid in chest cavity, 2 to 3 c. c.

Rabbit 928.—Fatal in three hours. Excess discolored serous fluid in abdomen containing oily globules. Moderate injection in intestines at points. Slight excess of fluid. Lungs slightly congested and slightly edematous.

Rabbit 929.—Died during night between the second and the thirteenth hour. Excess brownish serum in abdominal cavity. Small intestines show areas of marked injection. Lungs congested and somewhat edematous.

Rabbit 930.—Slightly indisposed on following day and normal thereafter. Appetite only slightly affected.

Rabbit 933.—Same as 930.

Feeding Gossypol Extract with Corn Meal and Molasses

An artificial cottonseed meal was made by pouring the concentrated ether extract of cottonseed kernels over corn meal. The daily feed for each of four rabbits was estimated to be equivalent to 30 gm. of cottonseed kernels, and for each of two others, 15 gm. Control animals were given corn meal and molasses. All the animals were supplied liberally with green feed (pea vines, cabbage, and collards) in the morning. In the afternoon (4 or 5 p. m.) they were given the various feeds mixed with molasses. The controls on corn meal and molasses did well, gained in weight, and need not be further mentioned. The gossypol extract proved very toxic. The animals receiving the equivalent of 30 gm. of cottonseed kernels refused to eat the cottonseed feed after the fifth day. They began to refuse green feed later, became sicker, and the last one died within 15 days. The two rabbits and a guinea pig receiving smaller doses were soon made sick. One rabbit and a guinea pig refused the feed thereafter, and the other rabbit died. See Table IV.

Table IV.—*Results of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig* [a]

DAILY FEED EQUIVALENT TO 30 GM. OF COTTONSEED KERNELS

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 941...	1,535	1,255	280	52	104	8	Died.
Rabbit 942...	1,605	1,250	355	54	108	12	Do.
Rabbit 943...	1,530	1,180	350	70	140	11.5	Do.
Rabbit 944...	2,095	1,595	500	71	142	15	Do.
Average...	1,691	1,320	371	62	124	11.7	Do.

[a] 1 gm. of the mixture of feed 318 and dry corn meal is equivalent to approximately 2 gm of cottonseed kernels.

TABLE IV.—*Result of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig*—Continued

DAILY FEED EQUIVALENT TO 15 GM. OF COTTONSEED KERNELS

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 953...	1,915	1,755	160	41	82	11	Died.
Rabbit 954...	1,790	1,740	50	80	160	20	Experiment discontinued.
Gossypol extract alone:							
Guinea pig A	770	650	120	34	68	29	Do.

POST-MORTEM OBSERVATIONS

Rabbit 941.—Reddish serum in abdominal cavity, 15 c. c. Cecum deeply injected. Liver congested. Lungs slightly congested and edematous. Conspicuous thrombus in right auricle.

Rabbit 942.—Excess abdominal fluid, 15 c. c. Hemorrhagic (inflamed) and ulcerated condition at pyloric end of small intestines. Large thrombus in right auricle.

Rabbit 943.—Slight excess of abdominal fluid. Large intestines had some hemorrhagic areas. Liver congested.

Rabbit 944.—Reddish serum in abdomen, 25 c. c. Serous membrane injected. Small intestines reddened. Small thrombi present. Death due to enteritis.

Rabbit 953.—Mesenteric blood vessels injected. Viscera practically normal. Liver much congested. Kidneys much congested.

Rabbit 954.—Experiment discontinued because animal refused to eat feed 318 after the eighth day. Subsequently put on precipitated gossypol.

Guinea pig A.—Experiment discontinued because animal refused to eat feed 318.

In order to ascertain the effect of a large dose, a large healthy rabbit (945) was taken from the control feed and given all of feed 318 that it would eat. It consumed 40 gm., equivalent to 80 gm. of kernels, on the first day and was made sick on the following day. When it began to recover on the fourth day it was given a small feed and died on the ninth day, having lost considerably in weight. The protocol of rabbit 945 is as follows:

September 23, p. m.—Ate 40 gm. of feed 318 with molasses.

September 24.—Appears sick; has diarrhea. Ate little green; refuses feed 318.

September 25.—Seems indisposed; refuses feed 318.

September 26.—Better; eats cabbage. Weight 2,700 gm. Given 15 gm. of feed 318 and 15 gm. of corn meal with molasses. Ate equivalent to 7 gm. of feed 318.

September 27, 28, and 29.—Eats pea vines readily.

September 30.—Refuses green; p. m., ate corn meal and molasses readily.

October 1, a. m.—Refuses green. Died ninth day about 3 p. m. Weight, 2,410 gm. Post-mortem examination showed considerable excess fluid in abdominal cavity.

TOXICITY OF PRECIPITATED GOSSYPOL

By the term "precipitated gossypol" we designate a product obtained from the gossypol extract. In securing the extract in larger quantities the oil was not entirely removed from the cottonseed kernels by several previous extractions with gasoline; hence, the gossypol extract contained considerable amounts of oil. The dark-red oily gossypol extract, after evaporation of the ethyl ether, was mixed with a large quantity of petroleum ether. Under some conditions a part of the gossypol precipitated in brown flocks, which could be separated easily by filtration. Under conditions of rapid precipitation these flocks would agglomerate and form a red resinous material. Both the light-brown powder and the red resinous material dissolved in ether very readily, giving a deep cherry-red solution.

Another artificial cottonseed meal was prepared by dissolving weighed quantities of precipitated gossypol in ether, pouring the solution over corn meal, and warming over a steam bath to drive off the ether. One gm. of precipitated gossypol was usually mixed with 50 gm. of corn meal. This proportion was based on the assumption that gossypol existed in cottonseed kernels to the extent of 2 per cent.

Our earlier estimate of 2 per cent appears to be too high. The largest yields of crystalline gossypol acetate secured from the extract were from 0.8 per cent to 1 per cent of the weight of the kernels. This probably represents nearly the entire amount present, as very little gossypol is dissolved by gasoline and little is left after ether extraction, judging by the slight toxicity of the residue.

Pouring the deep cherry-red solution over corn meal gave it a red color. When this was warmed over the steam bath, the color of the corn meal changed to a typical cottonseed-meal yellowish brown. No explanation is offered for this change; but it is evidently not due to oxidation, as the change begins at the bottom of the mixture, not at the surface.

This artificial meal was fed to six rabbits and proved fatal in every case. We had difficulty in getting them to eat it after having been once made sick.

Rabbit 954 was taken from feed 318 (gossypol extract) and offered corn meal and molasses containing 0.37 gm. of precipitated gossypol. It ate an equivalent of 0.3 gm. of the precipitated gossypol by the second day and seemed slightly indisposed. A week later it was again put on this feed, at the rate of 0.2 gm. daily. The quantity of gossypol eaten in the first six days was, per day, 0.2, 0.2, 0.17, 0.10, 0, and 0.05 gm. It ate none after this, but became sicker and died six days later.

Rabbit 961 ate 0.9 gm. of precipitated gossypol mixed with corn meal and molasses. It was apparently normal the next day, but refused cabbage on the third day. Thereafter it ate green feed well, but seemed to have no appetite for corn meal and molasses except when very hungry.

A week after recovery it was started on feed 319 (precipitated gossypol on corn meal). We planned to have it eat 0.3 gm. of gossypol daily. The first week 0.38 gm. of precipitated gossypol was eaten, the second week 0.67 gm., and only 0.60 gm. thereafter, a total of 1.65 gm. Death ensued after 19 days. The animal ate feed 319 sparingly and very irregularly.

A young rabbit (962) was fed similarly at the rate of 0.14 gm. a day. By weeks it ate, respectively, 0.97, 0.15, 0.15, and 0.34 gm. of precipitated gossypol. It was normal after the first week and died on the twenty-ninth day.

A guinea pig refused to do anything more than nibble feed 318 (gossypol extract), eating in 29 days only 34 gm. of the feed. It could not be induced to eat feed 319 (precipitated gossypol) any better, consuming only 1.13 gm. in 27 days. The autopsy showed that a mesenteric twist had cut off the blood supply of the last half or third of the intestines, so that death was not directly traceable to the feed.

Rabbit 949 was fed a large dose (1.44 gm.) of the precipitated gossypol mixed with corn meal and molasses. The next two days it suffered from diarrhea and refused to eat this feed, but it ate green feed. Thereafter it was given precipitated gossypol in small doses, but it usually refused all or part of this. Steadily losing weight, the animal died after 35 days, having eaten a total of 4.47 gm. of gossypol, inclusive of the large dose. The amounts eaten each week were, respectively, 2.08, 0.58, 0.50, and 0.68 gm.

Rabbit 937 had previously eaten the ether-extracted residue (feed 316) for 61 days and had increased in weight. Then, after several days on corn meal and molasses the rabbit was fed precipitated gossypol. We planned to feed 0.3 gm. a day, but only on three days did it eat this amount, usually refusing it entirely or in part. After 21 days a crystalline product was substituted for precipitated gossypol. The animal steadily decreased in weight and died after 33 days. The total amount of gossypol consumed was 2.52 gm. By weeks, 1.19, 0.27, 0.5, 0.57, and 0 gm. of gossypol were consumed. It ate practically nothing during the last 8 days. See Table V.

TABLE V.—*Results of feeding precipitated gossypol with corn meal and molasses (feed 319) to rabbits and guinea pigs*

Animal No.	Weight of animal.			Weight of precipitated gossypol eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
	Gm.	*Gm.*	*Gm.*			
Rabbit 954	1,740	1,275	465	1.02	13	Died.
Rabbit 961	1,830	1,435	395	1.22	19	Do.
Rabbit 962	630	465	165	1.62	29	Do.
Guinea pig A	660	565	95	1.13	27	Do.
Rabbit 949	2,375	1,702	673	[a]4.47	35	Do.
Rabbit 937	2,890	1,925	965	2.50	33	Do.

[a] This quantity (4.47 gm.) includes a large dose of 1.44 gm. which evidently passed the bowel quickly.

POST-MORTEM OBSERVATIONS

Rabbit 954.—Excess fluid in abdominal cavity. Serous membrane in icteric condition.

Rabbit 961.—Large excess abdominal fluid. Small intestines show enteritis. Blood vessels congested.

Rabbit 962.—Large excess abdominal fluid. Small intestines inflamed and hemorrhagic. Small thrombus in right heart.

Guinea pig A.—Evidently died from mesenteric twist (convolvulus) in intestines. Posterior third greatly inflamed. Lungs congested and edematous.

Rabbit 949.—Slight excess of abdominal fluid. Small intestines conspicuously inflamed. Large pericardial abscess present. Enteritis.

Rabbit 937.—Slight excess abdominal fluid. Small intestines irritated throughout. Conspicuous thrombi in heart. Lungs congested and edematous.

TOXICITY OF CRYSTALLINE GOSSYPOL "ACETATE"

Crystalline gossypol "acetate" was obtained from a gossypol extract by the action of glacial acetic acid, which caused a slow deposition of yellow crystals. We have designated this substance as an "acetate," although the acetic acid present is not firmly bound.[1] The product corresponded in general properties to Marchlewski's gossypol. It was administered intraperitoneally to four rabbits, proving fatal, and was fed daily to eight rabbits. It made all of them sick. One died from secondary causes. Two refused to eat the feed after 5 and 15 days, respectively, and five died within from 13 to 55 days, having eaten from 0.35 to 2.53 gm. of crystalline gossypol "acetate."

Intraperitoneal Injection of Crystalline Gossypol "Acetate"

We dissolved 1.2 gm. of gossypol "acetate" in ether and mixed the solution with 16 c. c. of cottonseed oil. The ether was evaporated by heating over a steam bath. This was given intraperitoneally to two rabbits of about 1,100 gm. weight so that each rabbit received from 0.5 to 0.55 gm. of gossypol "acetate." Both animals died and were cold in six hours. The autopsy showed a considerable portion of the dose in the abdominal cavity, so that much more than a lethal dose was given.

About 3 gm. of a yellow, crudely crystalline product similar to that which was injected in 0.5 gm. doses to rabbits 955 and 956 was recrystallized as follows: The material was dissolved in hot alcohol and heated to boiling, then 50 per cent of acetic acid was added until the liquid became slightly turbid. This mixture was again heated to the boiling point and allowed to cool. Most of the substance separated in yellow, flat, pointed crystals, about 0.1 to 0.5 mm. long, which melted with darkening at about 178° C.

[1] The term "acetate" is arbitrarily used. Gossypol crystallizes from glacial acetic acid and even from quite dilute acetic acid with a molecule of acetic acid, which is not removed by long boiling with water or by heating to 115° to 120°. Its presence thus escaped our attention as it did Marchlewski's. It is entirely improbable that a small amount of acetic acid modifies in any way the physiological action of gossypol. See "Results of feeding precipitated gossypol."

To prepare the injection, 0.7 gm. of this substance was dissolved in ether and the ethereal solution mixed with 20 c. c. of purified cottonseed oil. The clear reddish yellow solution was warmed over steam until it had not the slightest odor of ether. This was then injected in doses of 10 c. c. into two rabbits, 963 and 964, weighing 1,560 and 1,485 gm., respectively. In a few minutes the rabbits became very uneasy and then passed into a sort of stupor. Rabbit 963 died in 3.5 hours and 964 in 4.5 hours. The death of rabbit 964 was witnessed. Shortly before death it toppled over on its side, had several convulsions, gasped several times, squealed, and died.

In these cases, as in the previous one, there was considerable injecta left in the abdominal cavity. See Table VI.

TABLE VI.—*Result of administering crystalline gossypol "acetate" intraperitoneally in cottonseed oil to rabbits*

CRYSTALLINE GOSSYPOL "ACETATE"

Rabbit No.	Initial weight of rabbit.	Weight of gossypol.	Dose volume.	Weight of gossypol per kilo of body weight.	Result.
	Gm.	*Gm.*	*C. c.*	*Gm.*	
955	1,115	0.55	8	0.493	Died.
956	1,180	.55	8	.466	Do.

RECRYSTALLIZED GOSSYPOL "ACETATE"

963	1,560	0.35	10	0.244	Died.
964	1,485	.35	10	.235	Do.

POST-MORTEM OBSERVATIONS

Rabbits 955 and 956.—Dead and cold after six hours. Apparent nonabsorption of much of the injection. Excess of fluid. Peritoneum stained brown. Visceral blood vessels slightly injected.

Rabbit 963.—Died in convulsions. Part of injecta present as oily globules. Serum present also. Serous membrane stained yellow.

Rabbit 964.—Same as 963, except small intestines were rather markedly injected.

FEEDING CRYSTALLINE GOSSYPOL "ACETATE" TO RABBITS

Crystalline gossypol "acetate" with corn meal and molasses (feed 319) was fed to rabbit 965. The feed was refused on the fourth day, after which it was not further given. Only on the first day did the animal eat the entire amount fed. After eating 0.3 gm. of crystallized gossypol "acetate," it had a bad diarrhea and little appetite for green feed the next day. The protocol was as follows:

December 15, first day.—Ate 0.3 gm. with corn meal and molasses; weight, 2,340 gm.

December 16, second day.—Bad diarrhea, and eats little green feed.

December 16, p. m.—Ate 0.2 gm. of gossypol.
December 17, a. m.—Ate green feed well.
December 17, p. m.—Ate 0.17 gm. of gossypol.
December 18, a. m.—Ate green feed well.
December 18, p. m.—Refused to eat the "doped" food.
December 19, a. m.—Slightly sick; eats green feed moderately.
December 19, p. m.—Refused to eat corn meal and molasses, but ate green feed. Amount eaten, 0.67 gm.; final weight, 2,140 gm.; loss, 200 gm.
December 20 to December 31.—Ate green and corn meal and molasses; regained normal health.

Rabbit 951, weight 1,800 gm., which had previously stood two long feeding periods on ether-extracted cottonseed kernels, was fed crystalline gossypol "acetate." It ate 0.6 gm. in the first four days and then became sick, refusing all feed. On the tenth day it weighed 1,605 gm. From then till the twenty-eighth day, on which it died, it ate 0.32 gm. Weight about 1,170 gm.

Post-mortem observations: Teaspoonful excess in abdomen. Moderate injection of serous membranes. Some hemorrhagic areas in stomach. Mesenteric blood vessels more or less injected. Small thrombus in heart.

Rabbit 965A ate the same preparation of gossypol mixed with corn meal and molasses. It ate, by weeks, 0.64, 0.08, 0.5, 0.37, 0.07, 0.80, 0.31, and 0 gm.; total, 2.53 gm. This was a large healthy rabbit at the beginning. The post-mortem examination showed a slight excess of fluid in the abdominal cavity and serous membranes highly congested.

A new lot of rabbits was secured from a supply house in Washington, D. C. These rabbits were not as healthy and resistant as could be desired, some evidently having been used before in experimental work.

Rabbits 974, 976, 977, and 972 were given the same gossypol feed. Rabbit 974 ate 0.33 gm. of gossypol with corn meal and molasses. The next day it had a very bad diarrhea, which continued all day. It ate no green feed and only a little gossypol feed for the next four days, after which gossypol was withdrawn from the feed. On the nineteenth day it had not entirely recovered from the effects of eating 0.47 gm. of gossypol during the first five days. Loss in weight during 15 days, 330 gm.

Rabbit 976 was fed 0.25 gm. of gossypol. It had diarrhea the next day and no appetite. The third day it ate 0.05 gm.; fourth day, 0.09 gm.; fifth day, 0.01 gm.; and afterwards refused the gossypol feed. It lost in weight steadily until death, on the fifteenth day. Gossypol eaten, 0.40 gm. Loss in weight, 475 gm.

Rabbit 977 was fed like 976, with approximately the same effect. It died on the thirteenth day. Gossypol eaten, 0.35 gm. Loss in weight, 580 gm.

Rabbit 972 ate 0.55 gm. of gossypol and died in 13 days.

These last three rabbits were fed on a product which was somewhat darker in color than the gossypol given rabbit 974. The gossypol tends to take on a greenish or brown tinge under some conditions of prepara-

tion. Gossypol from old seeds is greenish. The post-mortem examination showed considerable irritation in the small intestines of these rabbits.

EFFECT OF SMALL DOSES OF CRYSTALLINE GOSSYPOL "ACETATE"

Rabbit 978, weight 2,100 gm., was fed on recrystallized gossypol "acetate" at the rate of 0.05 gm. daily mixed with corn meal and molasses. After one week it began to show a diminished appetite for the feed. On the nineteenth day it was given a double dose by mistake, and for two weeks thereafter showed a very poor appetite for the feed. At that time it weighed 1,820 gm.

Its record by weeks is as follows:

Quantity of gossypol eaten.....gm.. 0.34; 0.33; 0.335; 0.25; 0.25; 0.32; 0.125.

Weight of rabbit.....gm.. 2,045; 2,010; 2,070; 1,820; 1,930; 1,730.

Total quantity of gossypol eaten.....gm.. 1.95.

The animal died after 51 days. The post-mortem examination showed that a convolvulus had set up a necrotic condition in the intestine. Whether the feed was contributory to this condition we are unable to say. See Table VII.

TABLE VII.—*Results of feeding crystalline gossypol "acetate" (feed 319) to rabbits*

Feed and rabbit No.	Weight of rabbit.			Weight of gossypol "acetate" eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
Gossypol "acetate":	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
965	2,340	2,140	200	0.67	5	Made sick.
951	1,800	1,170	630	.92	28	Died.
965A	2,265	1,600	665	2.53	55	Do.
Recrystallized gossypol "acetate":						
974	1,670	1,340	330	.47	15	Made sick.
976	1,670	1,195	475	.40	15	Died.
977	1,825	1,245	580	.35	13	Do.
972	1,425	1,115	310	.55	13	Do.
Gossypol "acetate" fed in small doses (0.05 gm. per day):						
978	2,100	1,730	380	1.95	51	Died from secondary causes.

FEEDING GOSSYPOL "ACETATE" TO FOWLS [1]

Two cockerels (986 and 987) previously fed on cottonseed meal to study the symptoms, were started on gossypol. Powdered gossypol in 0.3 gm. doses was fed, followed by a little water. On the fourth

[1] This experiment was carried on under the supervision of Dr. B. F. Kaupp, Pathologist, of the Poultry Division, North Carolina Agricultural Experiment Station.

day cockerel 986 had fallen off in weight, and his appetite was only fairly good. On the sixth day his digestion was poor, his crop being full of food. The bird steadily lost in weight until death on the sixteenth day, dropping from 3 to 2 pounds in weight. The bird was given 4.1 gm. of gossypol, at least 0.5 gm. of which was found in the crop after death.

The post-mortem examination showed extreme emaciation. Food in crop for a number of days; indications that gossypol interferes with the nervous mechanism of digestion. Diarrhea, the contents being fluid in rectum only. Semisolid in other portions. An absence of visible lesions.

Cockerel 987, slightly larger than 986, reacted in quite the same manner as 986 to administrations of gossypol. He steadily wasted away, falling from 3 pounds 8 ounces to 2 pounds 3 ounces, and died on the twenty-sixth day. Amount of gossypol fed, 5 gm.

The post-mortem examination showed extreme emaciation. Testes, spleen, gizzard, and other organs to a certain extent in a state of absorption.

Of chief interest to us was a statement by Dr. Kaupp to the effect that the gossypol produced the same results as cottonseed meal.

A healthy pullet (989) was started on gossypol. On the fourth day her digestion was affected. Nine doses of 0.3 gm. each in a period of 20 days were sufficient to cause her to refuse all feed and to waste away. She died on the thirty-sixth day, weight 1.5 pounds, just half the initial weight. Dr. Kaupp reported that "the autopsy revealed nothing beyond extreme emaciation." See Table VIII.

TABLE VIII.—*Results of feeding gossypol "acetate" to fowls*

Fowl No.	Weight of fowl.			Weight of gossypol "acetate" eaten.	Death occurred in—
	Initial.	Final.	Loss.		
	Pounds.	*Pounds.*	*Pounds.*	*Gm.*	*Days.*
986	3.0	2.0	1.0	4.6	16
987	3.5	2.2	1.3	5.0	26
989	3.0	1.5	1.5	2.7	36

FEEDING GOSSYPOL "ACETATE" TO A PIG

Pig 989, weighing 21 pounds, was fed corn meal and molasses. He ate with relish. About 5 p. m. he was given 3 gm. of crystalline gossypol "acetate" mixed with 80 gm. of corn meal and molasses, the whole feed weighing about 125 gm. He ate all but a small part. The next morning he had little appetite. In the afternoon he was given 1 gm. of gossypol on corn meal and molasses, most of which was left on the following morning. The remainder was made into slop. He ate part of this. On the afternoon of the same day he vomited; the following morning he appeared sick. We were unable to continue this experiment.

TOXICITY OF GOSSYPOL EXTRACT FREED OF GOSSYPOL (FEED 340)

Gossypol extract was treated with acetic acid for the preparation of gossypol "acetate," as previously described. The precipitate contained most of the gossypol. The filtrate, which contained only a small amount of it, was mixed with corn meal and dried. The extract, thus practically freed of gossypol, was fed to two rabbits in very large amounts and produced no symptoms of poisoning in either.

The rabbits weighed 1,995 and 1,986 gm., respectively. Each was fed the extract from 500 gm. of kernels during five days, the daily amounts for the first two days corresponding to 130 gm. each and for the three other days, 90 gm. each. No rabbit could have eaten within this short period without fatal results such a large amount of kernels or the gossypol from them.

TOXICITY OF OXIDIZED GOSSYPOL (FEED 338)

Withers and Ray (1913b) noted that the toxicity of cottonseed meal could be destroyed by boiling with alcoholic caustic soda. The alkaline alcoholic filtrate from this treatment was also found to be nontoxic, owing to the oxidation of the phenolic gossypol to an organic acid. To ascertain the correctness of this view, weighed amounts of recrystallized gossypol dissolved in alcohol were treated with dilute caustic soda. The solution was exposed to air overnight, made slightly acid with hydrochloric acid, and evaporated to dryness. The residue was mixed with corn meal and molasses for feeding. The substance had a pronounced bitter taste. Two small rabbits ate the oxidation product, equivalent to 3 gm. of gossypol apiece, in the course of 16 days without the slightest sign of being affected thereby. See Table IX.

TABLE IX.—*Result of feeding oxidized gossypol to rabbits* [a]

Rabbit No.	Weight of rabbit.			Equivalent in gossypol of feed eaten.
	Second day.	Fifteenth day.	Gain.	
	Gm.	*Gm.*	*Gm.*	*Gm.*
983	1,280	1,420	160	3
984	850	1,065	215	3

[a] On 4 days out of the 16 oxidized gossypol was not fed.

TOXICITY OF KERNELS WITH GOSSYPOL INCOMPLETELY EXTRACTED

ETHER-EXTRACTED KERNELS (FEED 316)

Decorticated cotton seeds were secured from Charlotte, N. C. They were sifted to remove as much lint and hulls as possible. The kernels were then ground in a mill and sifted through an 18- to 20-mesh sieve and

extracted for five to eight hours with ethyl ether in a filter-paper thimble in a large Soxhlet apparatus.[a] After extraction the residual ether was evaporated and the kernels sifted through a 1-mm. sieve. They were either heated for an hour or so over a steam bath or dried in the air.

Sixteen rabbits and two guinea pigs were fed upon ether-extracted kernels. One of the rabbits had its back broken on the fifteenth day and was chloroformed. It showed none of the usual symptoms of cottonseed-meal feeding. Nine of the animals (Table X, part 1) died in from 19 to 75 days and 8 (Table X, part 3) were alive and normal at the end of the feeding experiments, which ranged from 42 to 71 days. Calculated to the average daily equivalent of kernels per kilogram of initial live weight, 9.4 gm. of ether-extracted kernels proved lethal to nine rabbits after 45 days, while 11.5 gm. did not prove lethal to eight others after 52 days. The ether-extracted kernels are therefore much lower in toxicity than cotton seed meal, of which a daily feed of 6.5 gm. per kilogram for 13 days was found lethal by Withers and Brewster (1913).

In view of the strikingly positive results obtained with ether extract and gossypol isolated therefrom, it was naturally expected that the ether-extracted kernels would prove nontoxic. With death resulting to only 9 out of 17 animals, and then not until after an average of 45 days, it is not unlikely that if the ether-extracted kernels had been fed in as small quantities as the cottonseed meal (6.5 instead of 9.4 gm.) they would have proved practically nontoxic, as anticipated.

The thoroughness of extraction is very important, as shown by the fact that kernels through which ether had only percolated proved toxic in from 11 to 14 days (Table X, part 2), while the average lethal period for kernels extracted from five to eight hours (Table X, part 1) was 45 days, or more than three times as long (Table X).

TABLE X.—*Results of feeding ether-extracted cottonseed kernels (feed 316) to rabbits*

PART 1

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
940	2,100	1,655	−455	348	522	14	35	Died.
935	1,380	1,500	+120	568	852	11	75	Do.
952	1,480	1,678	+198	251	373	20	19	Do.
947	1,875	1,576	−300	605	909	17	53	Do.
958	1,430	1,318	−112	494	741	15	52	Do.
959	2,560	2,165	−395	882	1,323	21	64	Do.
Guinea pig B (second period)[b]	610	535	− 85	199	300	9	34	Do.
970	1,515	1,670	+155	285	420	21	20	Do.
974	1,510	1,525	+ 15	458	687	14	50	Do.

[a] Before the ether extraction the ground kernels were extracted with petroleum ether or gasoline in case it was desired to work up the ether extract for gossypol.

[b] Rabbit 651 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

TABLE X.—*Results of feeding ether-extracted cotton seed kernels (feed 316) to rabbits*—Continued

PART 2

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
939	1,510	1,255	−255	[a] 89	133	12	11	Died.
938	1,930	1,405	−525	[a] 70	105	8	13	Do.
936	1,415	955	−460	[a] 69	103	7	14	Do.

PART 3

937	2,630	2,900	+270	1,013	1,520	25	61	Lived.
951 (first period)[b]	1,800	1,990	+190	609	914	22	42	Do.
951 (second period) [b]	1,940	1,790	−150	539	810	15	53	Do.
957	1,650	1,835	+185	627	942	21	46	Do.
Guinea pig B (first period)[b]	620	685	+ 65	320	480	10	50	Do.
960	2,040	2,195	+155	198	300	20	15	Chloroformed.
969	1,475	1,797	+322	798	1,197	16	71	Lived.
981	1,890	2,230	+340	880	1,320	30	44	Do.
985	1,315	1,730	+415	615	923	18	51	Do.

[a] Feed percolated only with ether.
[b] Rabbit 951 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

Rabbits 939, 938, 936, and 940.—The post-mortem examination showed symptoms resembling cottonseed-meal poisoning.

Rabbit 935.—When this animal had recovered from the effects of the incompletely extracted kernels, it weighed 1,030 gm. It ate 647 gm. in 49 days and then weighed 1,600 gm. It died on the seventy-fifth day, showing symptoms other than those common to cottonseed-meal poisoning.

Rabbit 937.—Slightly off its feed in the middle of the experiment, but was in perfect condition when this feed was discontinued.

Rabbit 952.—Post-mortem examination showed a small amount of excess abdominal fluid and the small intestines considerably congested. Death due to enteritis. Symptoms of cottonseed-meal poisoning.

Rabbit 951 (first period).—Kept in good condition most of the time.

Rabbit 951 (second period).—Somewhat affected by feed. Ate but lightly at end.

Rabbit 947.—About 40 c. c. excess serous fluid in abdominal cavity. Considerable necrosis had set up.

Rabbit 957.—Perfectly well at the end of the experiment and remained so during the subsequent three weeks. It acquired no "immunity" toward cottonseed poisoning, however. See data on rabbit 957 on feed 290.

Rabbit 958.—Put on this feed after being made sick on unextracted kernels (feed 290). Post-mortem examination showed about 15 c. c. excess serous fluid in abdomen; small intestines markedly injected with slight hemorrhagic areas; liver congested; large abscess in submaxillary lymphatic glands.

Rabbit 959.—Began to be affected on the forty-seventh day, having gained up to this date. The post-mortem examination showed excess bloody serum in abdominal cavity; large amount of serum present with coagulated fibrin; serous membranes congested.

Guinea pig B.—In perfectly normal health at the end of first feeding period. Died in second experiment, showing much irritation in intestines.

Rabbit 960.—Broke its back accidentally and was chloroformed. Its case is of interest in that the autopsy showed no pathological lesions in the time usually required to kill an animal with cottonseed meal.

Rabbits 969 and 970.—Had previously been on the alcoholic extract (feed 330) for 26 days without ill effects.

ARE THE BAD EFFECTS OF FEED 316 DUE TO GOSSYPOL?

Feed 316 is of a pale-yellow color. Moistened with ether and examined through a lens, numerous black specks are seen, as in the unextracted kernels. These represent the gossypol glands, the contents of which have in part been removed by ether. Sometimes these glands have become separated from the seed tissue and can be examined individually. They dissolve in concentrated sulphuric acid with a red color, indicating gossypol. On warming a gram or so of the extracted kernels with alcoholic potash and shaking, a darkening in shade with a suggestion of a purple color takes place in the supernatant liquid. This is characteristic of gossypol, the depth of color depending upon the amount of gossypol. When the alcoholic alkali first touches the particles, they turn several shades deeper to a yellow that matches the color of cottonseed meal very closely. This is also characteristic of gossypol. On the addition of acid the former light-yellow color returns.

If the extracted kernels are allowed to soak in water for a short while, a substance dissolves which gives the liquid a reddish violet color. This is probably due to an oxidation product of gossypol. The coloration is quite permanent.[1]

These experiments show that gossypol or oxidation products of gossypol or possibly other similar substances (see Power and Browning, 1914, p. 420) are still present in this residue after the long-continued ether extraction.

The fact that gossypol is not completely extracted by ether, although very soluble in it, may be due to its being held mechanically in impervious cells, being fixed dye like in the tissue, or being in the form of an insoluble metallic salt.

Therefore, it seems to us that even the slight toxicity of the residue after ether extraction is due to its gossypol content. (See data on rabbit 978, Table VII, p. 276.)

TOXICITY OF KERNELS PRACTICALLY GOSSYPOL-FREE

Ether-Alcohol-Extracted Kernels (Feed 331)

In order to determine whether it were possible by extraction with solvents to prepare a cottonseed feed which would not produce any bad results with rabbits, the ground kernels were extracted first with gasoline to remove oil, etc., then with ether in a large separatory funnel until the percolate was of a very faint-yellow color. The residue was

[1] An attempt will be made to correlate this observation with the red sap (anthocyan?) of certain species of Gossypium.

removed and boiled in a large flask with alcohol. The first alcoholic extracts were quite highly colored. The extraction was repeated until a filtrate was obtained which possessed only a pale-yellow color.

The ether-alcohol-extracted kernels were fed daily to three rabbits for from 72 to 105 days in amounts ranging from the equivalent of 15.2 to 24 gm. of kernels; at the end of the period the rabbits were normal and all had gained from 30 to 148 per cent of their initial weight and were still gaining.

The severe test that these rabbits endured is sufficient to show that a feed has been prepared which can be called practically nontoxic.

It also indicates that protein and organic phosphates (inosite phosphoric acid salts), which are present in the feed in larger amounts than in cottonseed meal, have very little, if anything, to do with cottonseed-meal poisoning.

TABLE XI.—*Results of feeding cottonseed kernels extracted with gasoline, ether, and alcohol (feed 331) to rabbits*

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.
	Initial.	Final.	Gain.		Total.	Daily.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
966	1,335	1,897	562	1,043	1,738	24	72
967	640	1,590	950	957	1,595	15.2	105
968	1,610	2,095	485	1,108	1,846	19.6	94

Rabbit 967 was slightly off its feed only on the fortieth and forty-first days, but recovered quickly and continued to gain. See Table XII.

Rabbit 968 was one of the lot of Belgian hares received from Washington, D. C., in rather poor health. It was started at the rate of 15 gm. daily, equivalent to 25 gm. of whole kernels. This proved too heavy feeding, for after two weeks the animal went off its feed for several days. The ration was then reduced (Table XII).

TABLE XII.—*Record of rabbits 967 and 968 on feed 331*

Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.	Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.
	Gm.	*Gm.*		*Gm.*	*Gm.*
Rabbit 967	.....	640	Rabbit 968	.....	1,610
1	62	705	1	140	1,525
2	70	845	2	85	[a] 1,410
3	73	930	3	81	[a] 1,460
4	93	.....	4	100	[a] 1,640
5	61	[a] 1,055	5	100	[a] 1,725
6	96	[a] 1,200	6	107	[a] 1,900
7	100	[a] 1,335	7	135	[a] 2,105
8	100	[a] 1,420	8	150	[a] 2,130
9	122	[a] 1,515	9	150	[a, b] 1,890
10	120	[a] 1,560	Last 4 days	60	2,095
Last 5 days	60	[a] 1,590			

[a] 10 gm. of corn meal was added daily to the feed.
[b] Loss in weight was due to the delivery of seven young rabbits.

TOXICITY OF AN ALCOHOLIC EXTRACT OF GASOLINE-ETHER-EXTRACTED KERNELS (FEED 330)

The solution obtained by treating gasoline-ether-extracted cottonseed kernels with hot alcohol was evaporated to a small volume over a water bath. The extract was about 10 to 12 per cent of the kernels. As the solution was concentrated, it separated into a yellowish layer (probably chiefly raffinose) and a reddish black resinous layer. The concentrated solution was mixed with corn meal, dried, and pulverized. This feed had a yellow-brown color and a very bitter taste. It was fed to two rabbits (969 and 970) in amounts equivalent to 50 gm. of cottonseed daily. It did not prove to be toxic, although the rabbits lost slightly in weight and frequently left part of their feed, possibly on account of its bitter taste. On the fourth day of feeding a slight diarrhea was noticed in both animals. They were quite normal after having been on the feed for 26 days, when it was discontinued (Table XIV).

TABLE XIV.—*Results of feeding an alcoholic extract of gasoline-ether-extracted cottonseed kernels (feed 330) to rabbits*

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.	Number of days fed.	Result.
	Initial.	Final.	Loss.				
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
969	1,530	1,475	55	243	1,000	26	Lived.
970	1,650	1,515	135	214	900	26	Do.

These two animals were then fed on the material from which the extract was obtained (see feed 316).

The presence of some gossypol due to the incomplete extraction by ether doubtless causes the slight toxicity of feed 316.

The nontoxicity of feed 330 may be explained on the assumption that the gossypol, extracted from feed 316 by alcohol, undergoes oxidation during the process of extraction or evaporation. This point needs further study (see feed 338).

Both the alcoholic extract and oxidized gossypol possess a bitter taste, whereas gossypol and gossypol "acetate" are tasteless and odorless.

ARE OTHER TOXIC SUBSTANCES PRESENT?

Although the feeding experiments show that gossypol is very poisonous, produces symptoms of cottonseed-meal poisoning, and affords a satisfactory explanation of the toxic properties of cottonseed meal, we do not claim to have made a complete study of the cottonseed from the standpoint of toxicity. The following problems are still unsolved:

(1) To exactly what extent does gossypol occur in cottonseed—i. e., in the petroleum extract and in the ether-extracted residue—and is gossypol the only toxic substance of like nature in the gossypol extract?

(2) To what extent, if any, do other toxic substances not related to gossypol contribute to the total action of cottonseed meal—i. e., are decomposition products and toxic alkaloids present in cottonseed meal? In this connection it may be stated that Friemann (1909) found an unidentified alkaloid in cottonseed meal, which caused paralysis of exposed frogs' hearts. Werenskiold (1897) obtained from cottonseed meal an alkaloid for which he proposed the name "gossypein." He also found betain and cholin. Withers and Fraps (1901, p. 81) state:

> Gossypein, if present in the sample tested, was present in very minute quantity. The filtrate from 363 grams cottonseed meal, ready for precipitation with phosphotungstic acid, was extracted with chloroform, and nitrogen was determined in the extract. It was equivalent to 0.008 per cent gossypein (calculated as cholin).

Withers and Ray (1913b) state:

> No evidence was found of the presence of toxic alkaloids in the feed, or of hydrocyanic acid in the feed or in the bodies of animals dead from eating cottonseed meal.

The fact that many solvents acting on *cottonseed meal* failed to remove the toxic substance suggests the possibility that in the manufacture of cottonseed meal the gossypol in the glands is fixed dyelike in the tissue of the seed, so that solvents like ether, in which gossypol is easily soluble, do not completely extract it. Gossypin is said to dye wool and silk (proteid materials). (See p. 265.) Again, some of the glands may be made impervious to the action of solvents by the mucilaginous substance surrounding the secretion. As is well known, cottonseed contains a large amount of raffinose (4 to 6 per cent). In the manufacture of the meal—e. g., in steaming—this may be partly dissolved and subsequently a film of this sugar deposited on the particles of meal. These factors must be considered with reference to the nonremoval of gossypol from the meal by solvents.

It may be noted that every gram of extracted residue represents at least 1.5 gm. of kernels. A ration of 15 gm. per day means that the animal eats all the protein and practically all the phosphorus of 22.5 gm. of seeds.

The residue (feed 316) is rich in nitrogen and ash. The values of nitrogen, protein, sulphur, and phosphorus in the ground kernels, and in feeds 316 and 331 are given in Table XV.

TABLE XV.—*Percentage of nitrogen, protein, sulphur, and phosphorus in ground cottonseed kernels and in feeds 316 and 331*

Feed.	Nitrogen.	Protein.	Sulphur.	Phosphorus.
Ground kernels	5.24	32.7	0.40	.....
Feed 316	8.6	53.7	.54	1.2
Feed 331	8.8	55.0	.....	.....

It is quite probable that the animal organism is able to take care of the large amount of proteins and phosphorus compounds, as may be inferred from the results of feed 331.

The latest published endeavor to ascribe the poisonous effects to a specific chemical substance was by Crawford (1910), whose experiments seemed to point to salts of pyrophosphoric acid.

The improbability of this conclusion was shown by Withers and Ray (1913a), of this Station, in feeding experiments. Cottonseed meal was extracted with ammonium citrate. This left an insignificant amount of phosphorus in the residue, which was almost as toxic as whole cottonseed meal.

Edgerton and Morris (1912) also conducted many feeding experiments with cottonseed and cottonseed meal. They fed sodium phosphate in large amounts and concluded that they had found "no evidence whatever to show that pyrophosphoric acid has anything to do with cottonseed-meal poisoning."

Rather (1912) also studied the phosphorus compounds of cottonseed meal and concluded that there was no evidence that the samples of cottonseed meal examined contained either pyrophosphoric acid or metaphosphoric acid. He also states (p. 16) that "the inorganic phosphorus (Forbes' method), in the samples of cottonseed meal examined was less than 5 per cent of the total phosphorus."

R. J. Anderson (1912, p. 5) isolated an inosite phosphoric acid very similar to phytic acid and made the following statement:

> The organic phosphoric acid of cottonseed meal gives all the reactions previously attributed to the presence of pyro- and meta-phosphoric acids. But the question whether or not it is also the toxic principle in cottonseed meal remains unanswered. Preliminary experiments carried out with the acid obtained from the purified barium salt on rabbits are not conclusive. Given in 0.5 and 1 gram doses, both the free acid and its potassium salt produced strong symptoms of distress, but after a few hours the animal regained their normal appearance. Larger doses passed through the bowel in a very short time and no definite symptoms developed.
>
> It is difficult to determine just what caused the toxicity of the preparations which were used in the experiments described by Crawford. It is evident that very impure substances were fed.

Since inosite phosphoric acids occur in numerous feeding stuffs other than cottonseed meal—e. g., wheat bran, corn, oats, barley—and since no suspicion of toxicity has occurred in these substances it seems highly improbable that the phosphoric acids in cottonseed meal have any significant action as toxic agents.

METHODS FOR REMOVING OR DIMINISHING THE TOXICITY OF COTTONSEED

Three methods have been proposed at the North Carolina Experiment Station and have been found effective for diminishing the toxicity of cottonseed kernels or cottonseed meal:

(1) Extraction of the kernels with ether (feed 316) or with ether and with alcohol (feed 331). By these methods gossypol is reduced to such a small amount that the residue is only slightly toxic (feed 316) or is nontoxic (feed 331).

(2) Treatment of the meal with an alcoholic solution of an alkali (Withers and Ray, 1913b). This treatment affords conditions for rapid oxidation, and oxidized gossypol has been found by us to be nontoxic (feed 338).

(3) Treatment of the meal with iron salts (Withers and Brewster, 1913) and Withers (1913). Treatment with iron salts is accompanied by some chemical action, as shown by the pronounced change in the color of the meal. The favorable physiological changes may be due to oxidation of the gossypol or to the formation of a more difficultly soluble compound. The oxidation may be due to the stimulating action of iron upon the oxidases of the animal body or to the direct action which ferric salts exert upon phenolic bodies. Ferrous sulphate forms an insoluble lake with gossypol. We have not yet studied it, but as Marchlewski (1899) found the lead salt so stable that it was not decomposed by hydrogen sulphid nor sulphuric acid, it is likely that the iron lake is very stable also.

The seed tissue surrounding the cells probably prevents the free action of reagents which would extract gossypol or render it physiologically inert. This constitutes the principal difficulty that must be overcome by the oil miller or stock feeder in rendering cottonseed meal nontoxic.

SUMMARY

(1) Gossypol, first isolated by Marchlewski from cottonseed oil and considered by him a prospective dyestuff, was extracted by us from cottonseed kernels and found to possess toxic properties.

(2) Cottonseed kernels were used as the initial material instead of cottonseed meal, because they yield gossypol more readily to solvents and are toxic to about the same extent.

(3) Ethyl ether was used as the solvent, the kernels having been extracted with gasoline to remove most of the oil. Evaporation of the ether leaves a crude product which we have designated "gossypol extract." A purer product, "precipitated gossypol," was obtained from the ethereal solution by the addition of gasoline, and a crystalline product, "gossypol 'acetate,'" by precipitation by acetic acid.

(4) Gossypol was fatal to rabbits when administered intraperitoneally in the form of gossypol extract or crystalline gossypol acetate, either when fed in one large dose in the form of gossypol extract or when fed in small daily doses in the form of gossypol extract, precipitated gossypol, or gossypol "acetate."

(5) Gossypol forms an oxidation product which is nontoxic.

(6) Cottonseed kernels are rendered less toxic by the partial extraction of gossypol and nontoxic by a more nearly complete extraction of it.

(7) Methods for rendering cottonseed kernels nontoxic depend upon extracting the gossypol or changing it to physiologically inert forms by oxidation or by precipitation.

(8) The smallest amount of gossypol administered intraperitoneally by us and found fatal to rabbits was 0.24 gm. of crystalline gossypol acetate per kilo of live weight.

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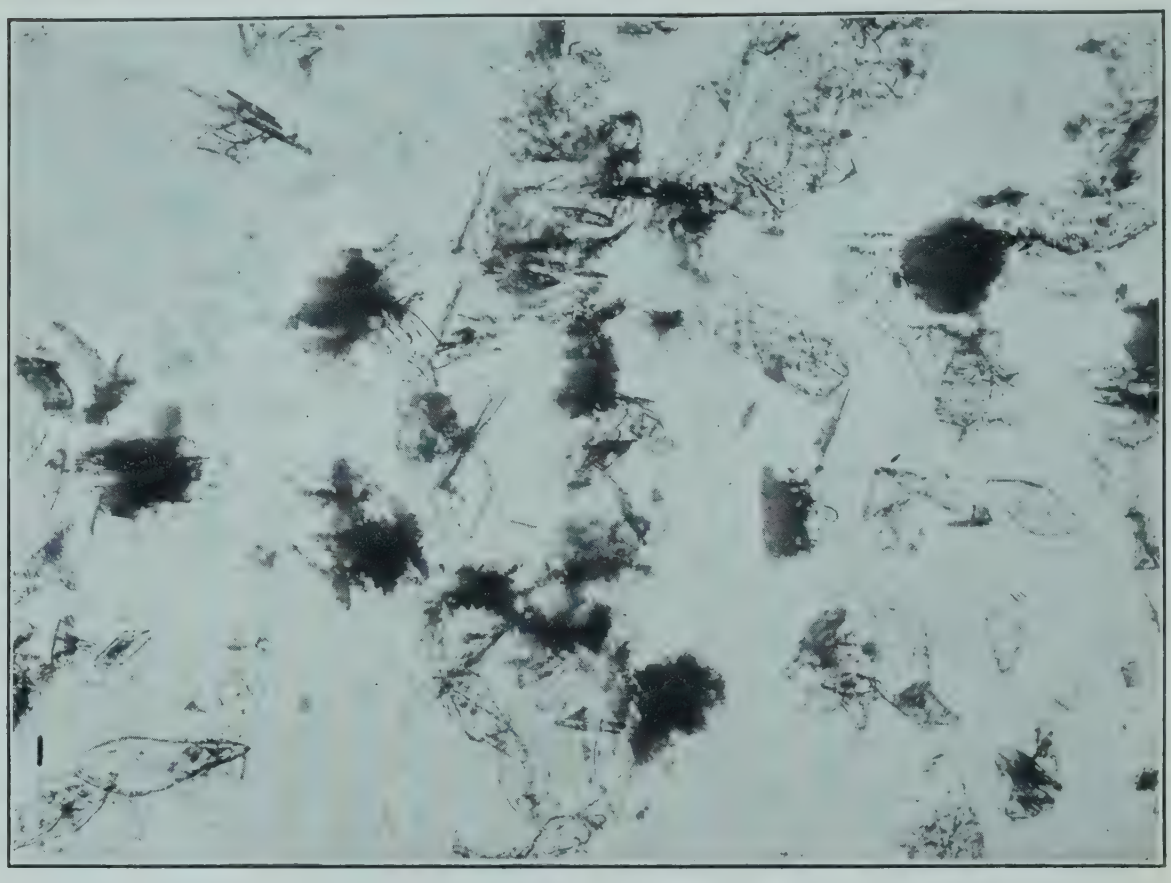
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TWO NEW HOSTS FOR PERIDERMIUM PYRIFORME

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Investigations in Forest Pathology, Bureau of Plant Industry

Peridermium pyriforme Peck, which is the æcial form of *Cronartium pyriforme* (Peck) Hedgc. and Long, was collected for the first time on *Pinus rigida* Mill. by the senior writer on June 16, 1915, near Essex Junction, Vt. (F. P. 17708).[1] This is the first collection which has been reported on this host. The senior writer had previously found the uredinial and telial forms in abundance in the same locality on *Comandra umbellata* (L.) Nutt. (F. P. 8655) on July 31, 1913. This find is important, since it may serve to clear up the mystery associated with the identity of the host in the case of the type specimen on *Pinus* spp.,[2] collected by Prof. J. B. Ellis (2040) in 1880, possibly near Newfield, N. J., Ellis not being certain as to the locality. Since the telial form was collected by Ellis (Ellis and Everhart, N. A. Fungi, No. 1082) near Newfield in 1879 and as *Pinus rigida* is the only native species of pine in this locality known to be attacked by the fungus, it is very probable that this species is the host of the type. In measurements and shape the spores of the writers' specimen agree with those of the type which the writers have examined at the herbarium of the State Museum at Albany, N. Y. The type specimen consists of a young pine twig whose bark closely resembles in color and markings that of *Pinus rigida*.

Mr. Roy G. Pierce, of this office, collected a number of specimens of *Peridermium pyriforme* on *Pinus divaricata* (Ait.) Du Mont de Cours (Pl. XXVII, fig. 1) in several localities near Cass Lake, Minn., during the month of June, 1915 (F. P. 18044, 18046, 18047, 18058, 18060, 18072, and 18076). So far as the writers know, only one specimen of the fungus has hitherto been reported on *Pinus divaricata*, and that was found by Mr. J. J. Davis in Douglas County, Wis. Mr. Pierce reported that the fungus was common where he collected it, and it is probably common also in other localities. He also found the uredinial form, *Cronartium pyriforme*, on July 11, 1915, on *Comandra umbellata* in the same locality as one of his previous collections of the æcial form.

The junior writer also has a specimen of this rust (F. P. 19440) on *Pinus divaricata* collected at Roscommon, Mich., by State Forester Marcus Schaaf. This specimen was sent in with *Peridermium cerebrum*, which on this host produces typical globular swellings, while *Peridermium pyriforme* causes the typical fusiform swellings. *Peridermium pyriforme*, however, does not always produce fusiform swellings, since the junior writer has recently received a specimen (F. P. 19437) on a 4-year-old

[1] "F. P."=Forest-Pathology Investigations number.

[2] Hedgcock, G. G., and Long, W. H. A disease of pines caused by Cronartium pyriforme. U. S. Dept. Agr. Bul. 247, p. 7. 1915.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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transplant of *Pinus* (*murrayana*) *contorta* Loud., collected at Roscommon, Mich., by Mr. Schaaf, which produced a globoid gall (Pl. XXVII, fig. 2) extending nearly around the attacked stem. This gall was 6 cm. in circumference and 2 cm. in diameter. Both above and below the gall were irregular lesions caused by *Peridermium comptoniae* (Arthur) Orton and Adams. The gall resembled so closely the swelling produced by *Peridermium cerebrum* that the junior writer thought it was this species until he examined it under the microscope, when he found the typical pyriform spores of *Peridermium pyriforme*.

In June, 1915, the junior writer received a fine specimen of *Peridermium pyriforme* (F. P. 19429) on *Pinus arizonica* Engelm., a 3- to 5-leaved pine (Pl. XXVII, fig. 3), collected by Ranger J. H. Woolsey in Jacobson's Canyon, Crook National Forest, Arizona. This is the first time this rust has been reported on this host. Many of the æcia of the specimen were very large and unusually prominent, owing to their marked extension beyond the bark. Some were over 2 cm. long and from 5 to 6 mm. in height. The galls were of the effused type and were from 40 to 50 cm. long. One of the branches attacked was about 2 inches in diameter where the lesions occurred. Its bark was very rough and exfoliated by the action of the fungus. The lesions had completely surrounded the two branches for a distance of from 20 to 30 cm., but had not yet killed them.

The writers have previously found *Peridermium pyriforme* only on pines having two to three needles in the leaf cluster,[2] and the occurrence of the fungus as now reported on *Pinus rigida* and *Pinus arizonica* is of interest, since it adds to the list of known hosts two pines of the group bearing three needles in a cluster. *Pinus rigida* has three needles and *Pinus arizonica* three to five needles.

It is now known that *Peridermium pyriforme* causes three forms of disease on pines; one with slight or no hypertrophy, common on *Pinus divaricata*, *Pinus pungens* Michx., and *Pinus ponderosa scopulorum* Engelm.; a second causing a fusiform or spindle-shaped swelling and found on *Pinus arizonica*, *Pinus* (*murrayana*) *contorta*, *Pinus divaricata*, *Pinus ponderosa* Laws., *Pinus ponderosa scopulorum* Engelm., and *Pinus rigida;* and a third form, causing the formation of globose galls (Pl. XXVII, fig. 2) now first reported on *Pinus* (*murrayana*) *contorta*.

Peridermium pyriforme, especially when weathered, superficially resembles *Peridermium comptoniae*, with which the senior writer found it associated near Essex Junction, Vt., where he found 1 specimen of the former and nearly 50 of the latter species. It is quite probable that this resemblance has frequently caused it to be overlooked by collectors wherever two species occur together and that a more careful search for *Peridermium pyriforme* will greatly extend the known range of the disease of pines caused by it. The spheroid galls of *Peridermium pyriforme* resemble very closely the spheroid galls of *Peridermium cerebrum* (Pl. XXVII, fig. 2); and unless the spores are examined, this form might be easily mistaken for the latter fungus.

[2] Hedgcock, G. G., and Long, W. H. Op. cit.

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PATHOGENICITY AND IDENTITY OF SCLEROTINIA LIBERTIANA AND SCLEROTINIA SMILACINA ON GINSENG

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INTRODUCTION

For a number of years two species of Sclerotinia have been recognized as probable causes of the rotting of ginseng roots (*Panax quinquefolia*), but the pathogenicity and identity of these fungi have not been proved by by inoculation experiments.

The purpose of this paper is (1) to report inoculation experiments establishing the pathogenicity of these organisms, and (2) to detail the experimental data and considerations on which the conclusions as to the identity of the two pathogens are based.

WHITE-ROT OF GINSENG

The white-rot of ginseng was first reported by Whetzel (1907, p. 89).[2] Sclerotia were found, but the identity of the fungus was not determined. Subsequent workers, Rankin (1910), Osner (1911), and Whetzel and Rosenbaum (1912, p. 34–45) have attributed the disease to *Sclerotinia libertiana* Fuckel. These writers based their observations on the association of the sclerotia of the fungus with the host and the general resemblance of the organism on the host and in culture to the widespread *Sclerotinia libertiana*. No inoculation experiments have been reported.

PATHOGENICITY

During the spring of 1913 the fungus was isolated from diseased ginseng roots grown at Newtown, Pa., Mentor, Ohio, and Edenville, Mich. The isolations were made by washing the roots, immersing them for 10 minutes in a solution of mercuric chlorid (1 to 1,000), peeling back a portion of the external tissues, and transferring small bits of tissue from the inside of the root to poured plates of hard potato agar. Pure cultures were obtained in the majority of cases from the first planting. In addition to the cultures isolated from ginseng, inoculations on healthy ginseng

[1] The writer is indebted for many suggestions to Dr. Donald Reddick, of Cornell University, under whose direction this work was done.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 297.

roots were also made with a culture of *Sclerotinia libertiana* obtained from lettuce from South Carolina. The procedure followed in the inoculations was as follows: Healthy ginseng plants with the tops still attached were selected and the soil carefully removed from one side of the root. By means of a flamed scalpel longitudinal cuts were made in the side of the root. These cuts were approximately one-fourth of an inch in length and about one-eighth in depth. A piece of agar containing mycelium from young cultures was inserted within these cuts and covered with soil. Check roots were treated in a similar manner.

During the summer inoculations were made as shown in Table I. The checks in every case remained healthy.

TABLE I.—*Results of the inoculation of ginseng with Sclerotinia libertiana from various sources*

Date.	Source of culture.	Number of roots inoculated.	Number of checks.	Percentage of infection.
July 14	*Sclerotinia libertiana* from South Carolina from lettuce	6	2	100
15	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	6	2	100
15	*Sclerotinia* sp. from Newtown, Pa., from ginseng.	8	4	100
15	*Sclerotinia* sp. from Edenville, Mich., from ginseng	6	2	83+
Aug. 1	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	4	1	100
1	*Sclerotinia libertiana* from South Carolina from lettuce	4	1	75

Plate XXVIII, figures 1 and 2, is reproduced from photographs of ginseng roots from two of the above series. Figure 1 shows a root inoculated with *Sclerotinia libertiana* isolated from lettuce. Figure 2 shows three roots (on the left) inoculated with a species of Sclerotinia isolated from ginseng.

Reisolations were made from the inoculations of July 15 and the fungus was again grown in pure culture. Inoculations made with the reisolated culture gave positive results.

Infection was evident in from three to seven days after inoculation. The root at the point of inoculation becomes soft and the rot spreads gradually in all directions, causing the entire root to beame soft and doughy. After the mycelium has penetrated throughout the tissues of the root, it forms tufts of cottony-white felt, in which large black sclerotia rapidly develop. Sclerotia on the outside of the root have in some cases developed within 10 days after the inoculations were made. When the inoculations are made near the crown of the root, the mycelium spreads to the stem, where it develops similar sclerotia on both the inside and the outside of the stem. The rapidity with which the disease progresses in the inoculted roots depends upon moisture conditions.

During a rainy period infection is evident within a much shorter time. All attempts to produce the disease without previously injuring the root gave negative results.

IDENTITY OF THE SPECIES

In order to further prove that the species of Sclerotinia from ginseng is identical with *Sclerotinia libertiana* Fuckel, a comparison was made with cultures from different sources. In addition to the four strains mentioned above, there was also used a pure culture isolated by Dr. Donald Reddick, of Cornell University, from celery. The comparison of the strains consisted in (1) growing the cultures on different media, both acid and alkaline; (2) production of apothecia, measurements of asci, ascospores, and a study of the manner of germination; (3) cross-inoculations on lettuce. These topics are briefly discussed in the following paragraphs.

GROWTH ON DIFFERENT MEDIA.—Cultures were made on potato agar, nutrient agar, bean plugs, ginseng stems, and Raulin's synthetic fluid. In the case of potato and nutrient agar both acid and alkaline media were used (± 10.5 Fuller's scale). On all the media the various strains made a good growth, but no differences were visible.

PRODUCTION OF APOTHECIA, ETC.—In order to obtain apothecia from the various strains, the sclerotia produced in pure culture were placed on sterile moist sand in dome-shaped preparation dishes. The sclerotia were covered with a very thin layer of the sand, and the dishes were placed on a shelf in front of a window. The time required for these apothecia to develop varied greatly, the limits being from three weeks to three months. The size of the apothecia likewise varied even in the case of sclerotia from the same strain and produced in the same test tube. However, the apothecia were alike in general appearance in all the strains. Plate XXVIII, figure 3, shows apothecia from the celery strain, and Plate XXVIII, figure 4, shows the same from the ginseng strain. A large number of measurements made of asci, paraphyses, and ascospores showed no marked variations, and agreed with the description of *Sclerotinia libertiana* Fuckel as given in Saccardo. In figure 1, *A*, is shown a camera-lucida drawing of asci, ascospores, and paraphyses from a fresh preparation of the Mentor strain.

Crushed pieces of apothecia were placed in drops of water in order to observe the ascospore germination. Within four hours after being placed in water the first signs of germination became visible. Figure 1, *B*, shows the ascospores within the asci, germinated by sending germ tubes directly through the walls of the ascus. No differences were noted in the germination of the spores from the different strains.

INOCULATIONS ON LETTUCE.—Mature lettuce plants were selected and inoculated with the various strains of the fungus. Inoculations were

made on injured and uninjured plants, which were then covered with bell jars for 4 days. At the end of 12 days most of the plants showed signs of rotting. Unlike the ginseng roots (Pl. XXVIII, figs. 1 and 2) previously discussed, infection occurred not only on the injured, but also on the uninjured plants.

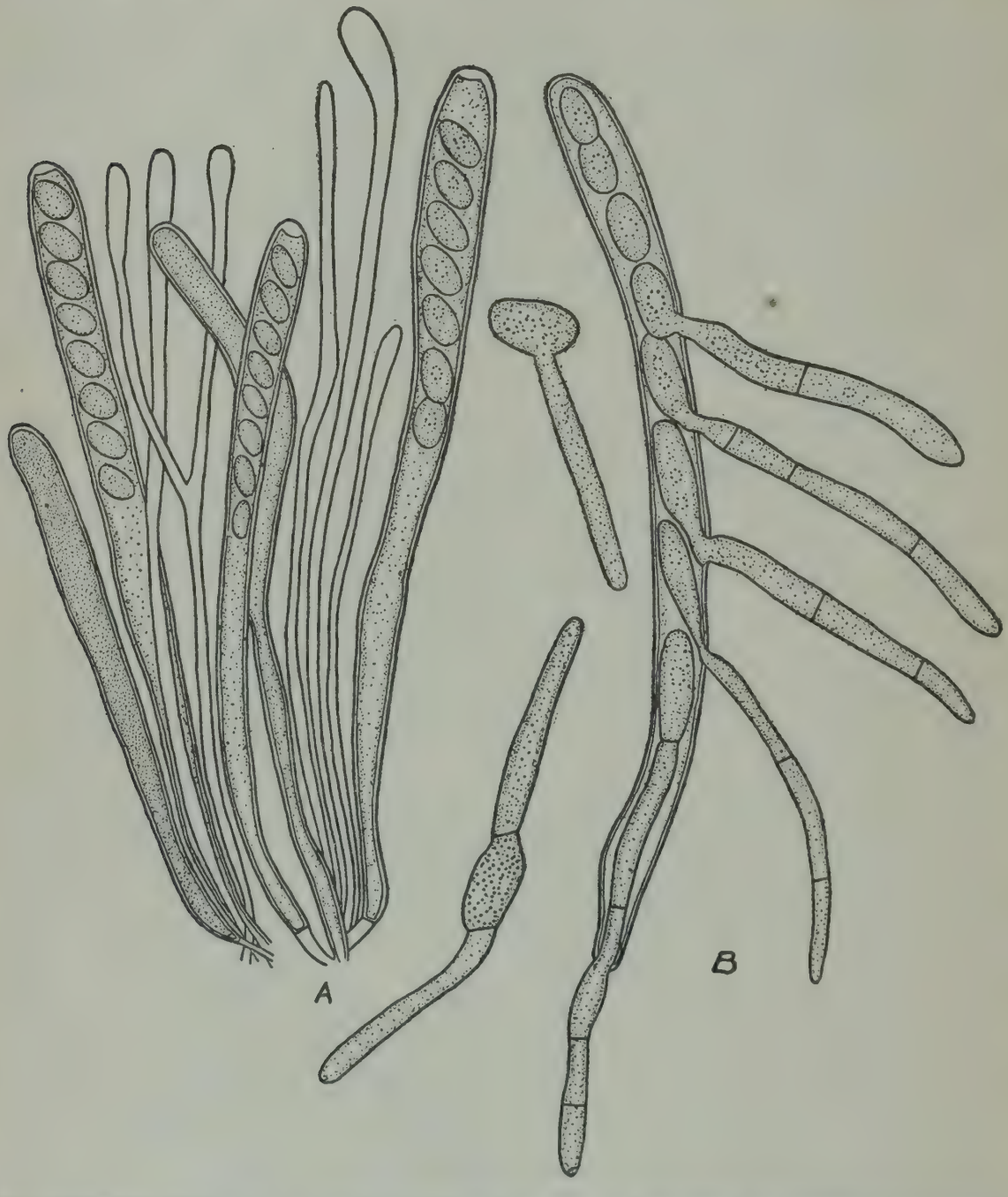

FIG. 1.—*Sclerotinia libertiana: A*, Camera-lucida drawing showing branched and unbranched paraphyses, asci, and ascospores; *B*, camera-lucida drawing showing methods of ascospore germination. Those within the asci germinate by sending germ tubes directly through the walls of the ascus.

BLACK-ROT OF GINSENG

Van Hook (1904, p. 181–182) first mentions a species of Sclerotinia as the cause of a black-rot of ginseng. Rankin (1912) reports the discovery of the apothecia and established a new specific name for the fungus. No inoculations were attempted, either on the ginseng roots or on other hosts known to be attacked by species of Sclerotinia closely allied to this one.

PATHOGENICITY

In the spring of 1912 the writer received a number of black-rotted roots from Wisconsin showing various stages of development of the disease. Isolations were made from these roots by making plantings from the inner tissues of the roots on poured plates of hard potato agar. The fungus was obtained in pure culture, where it produces a characteristic black growth.

Inoculations on healthy roots made at various times during the summer gave negative results, as would be expected from the nature of the fungus, since the disease always develops in beds during the winter. In October of the same year (1912) six roots were washed clean and inoculated by placing a piece of the agar pure culture in a small cut made in the tissues of the root. Three similar roots were injured and used as checks. All the roots were planted in soil which had never grown a crop of ginseng. The following March an examination of the roots showed the characteristic symptoms of the disease. Some were entirely black, while others were only partly blackened. The fungus was easily reisolated from these roots. Plate XXIX, figure 1, shows two inoculated roots, together with a check root. One of the inoculated roots is entirely black, while the second shows this black color only in part.

In October, 1913, inoculations were again made on ginseng roots. These roots were not injured, but the fungus was placed on the old stem scar. The next March the roots were black, as in the previous year. Reisolations were again made, and the fungus which was obtained produced the characteristic black growth.

IDENTITY OF THE SPECIES

The growth of the fungus in culture and the general behavior of this organism differed so greatly from the known species of Sclerotinia that it has always been an interesting question as to the source of the fungus which appeared in isolated gardens throughout the country. One plausible explanation is that the fungus, being associated with wild ginseng roots or with one of the common weeds, was brought in from the woods, as many growers make a practice of using leaf mold in preparing their beds. Since the fungus from the description resembled *Sclerotinia smilacina* Durand, it seemed advisable to determine whether the species of Sclerotinia on ginseng could produce a black-rot of the rhizome of *Smilacina* spp. and whether the two were also identical in other respects.

Inoculations on species of Smilacina.—In October, 1913, six rhizomes of *Smilacina racemosa* were inoculated with a pure culture of the black-rot fungus obtained from ginseng. The inoculations were made by slightly injuring the rhizome and inserting the mycelium of the fungus in the cut. Check plants were also injured. When examined the following March, the rhizomes showed the characteristic symptoms of black-rot

as exhibited by ginseng roots. The check plants remained healthy. Plate XXIX, figure 2, is a reproduction of a photograph of two of the inoculated and one check rhizome. Reisolations were made, and the fungus which was obtained resembled the original culture isolated from ginseng.

COMPARISON WITH TYPE SPECIMEN.—To determine further the relationship of the *Sclerotinia* sp. from ginseng to that on *Smilacina* spp., an examination was made of the type specimen of *Sclerotinia smilacina* Durand, deposited by Dr. Durand in the herbarium of the botany department of Cornell University. The specimens showed the black coloration as exhibited by the inoculated rhizomes of *Smilacina racemosa* as well as the ginseng roots.

Apothecia on ginseng are rare, and though attempts to produce them were made no success can be reported up to the present time. It is of interest, however, to compare the measurements as given in the original descriptions by Durand (1902, p. 462–463) and Rankin (1912) as shown in the following table:

Species.	Sclerotia.	Apothecia.	Asci.	Ascospores.
	Gm.	*Gm.*	*μ*	*μ*
Sclerotinia smilacina . .	0.1 by 0.2 to 2.	0.75 to 3 . .	120 to 140 by 6 to 8.	12 to 15 by 4 to 5.
Sclerotinia panacis	0.3 to 1	1.5 to 2.5 . .	125 to 137.5 by 6.4 to 6.5.	11.7 to 16 by 4.8 to 7.5.

Measurements made by the writer from the type material of these species have shown that the asci and ascospores are not to be distinguished either in form or size and agree with the measurements given above.

CONCLUSIONS

1. (A) The pathogenicity of *Sclerotinia* sp. causing the white-rot of ginseng has been established. (B) This species of Sclerotinia is identical with the *Sclerotinia libertiana* Fuckel occurring on lettuce, celery, and a number af other hosts.

2. (A) The pathogenicity of *Sclerotinia* sp. causing the black-rot of ginseng has been established. (B) A consideration of the following facts indicates that *Sclerotinia panacis* Rankin is identical with *Sclerotinia smilacina* Durand: (a) Inoculations with a species of Sclerotinia from ginseng on *Smilacina racemosa* gave positive results. (b) Measurements of asci and spores made by the writer from the type material of both species agree. There is a close agreement in all distinguishing characters, as given in the original description of the two species. (c) The lesions produced by the inoculations are similar on the two hosts and identical with those on diseased plants as they occur naturally.

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PLATE XXVIII

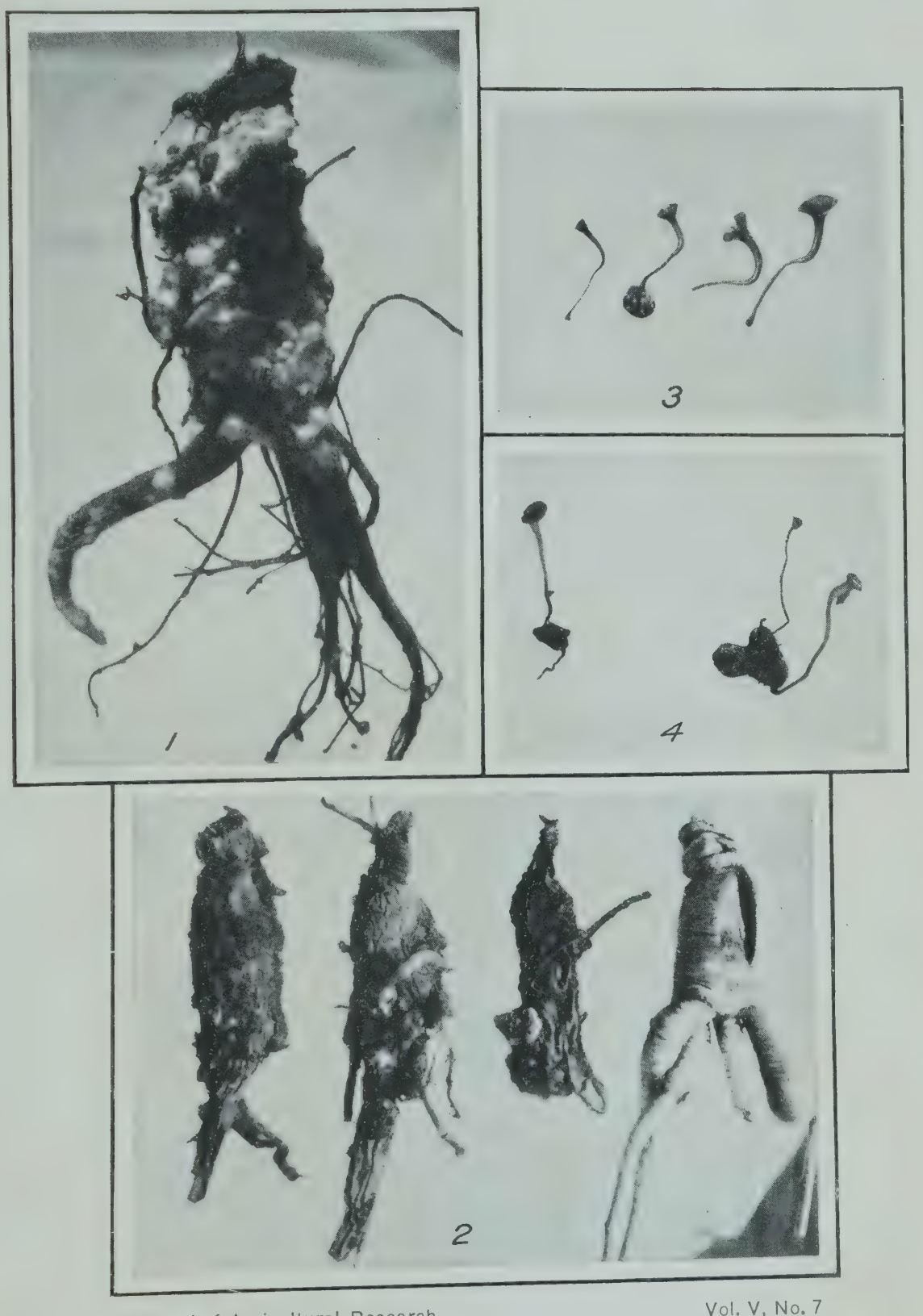
Sclerotinia libertiana:

Fig. 1.—Root inoculated with *Sclerotinia libertiana* from lettuce. Note the white mycelial felt and the production of sclerotia.

Fig. 2.—Three roots (on left) inoculated with *Sclerotinia* sp. from ginseng. Healthy check root (on right).

Fig. 3.—Apothecia from sclerotia from celery strain.

Fig. 4.—Apothecia from sclerotia from ginseng strain.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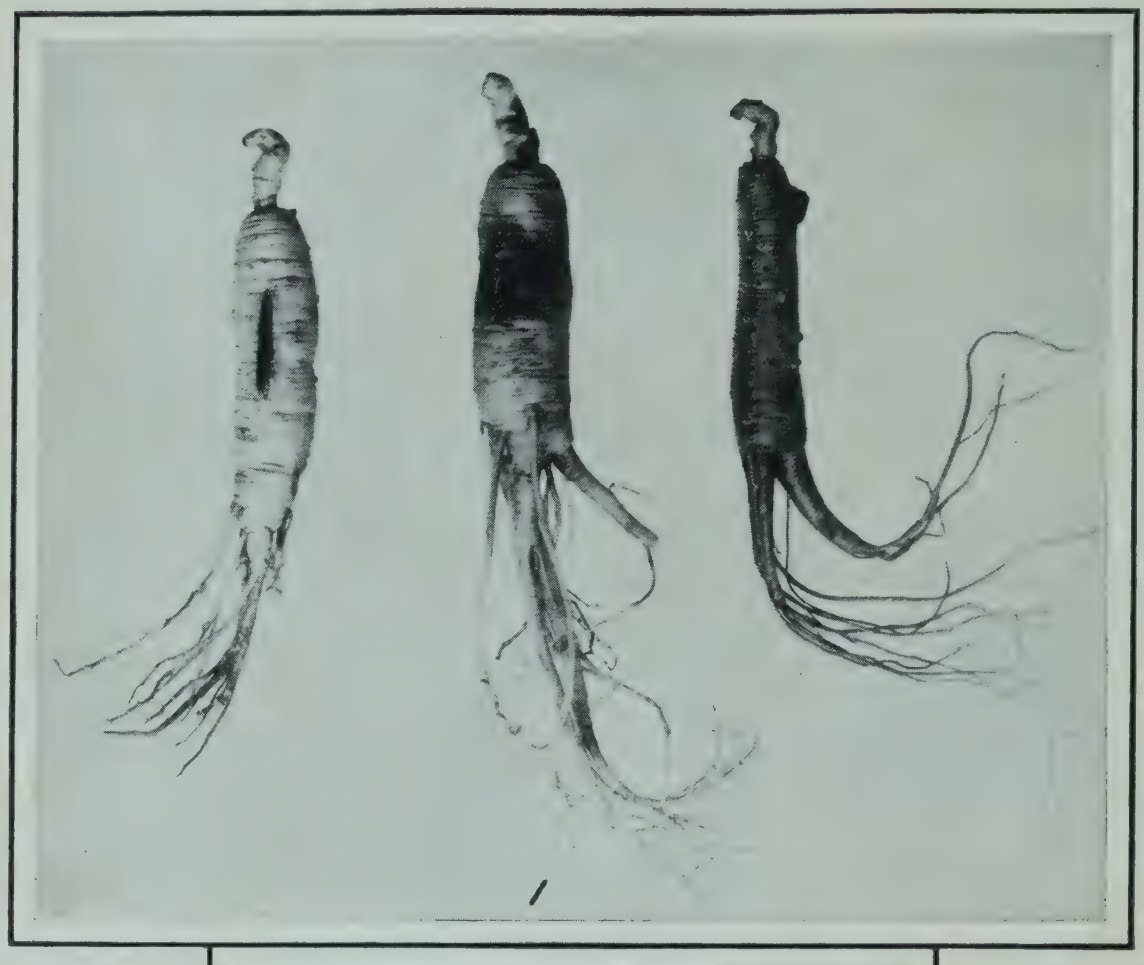

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., NOVEMBER 22, 1915 No. 8

AN IMPROVED RESPIRATION CALORIMETER FOR USE IN EXPERIMENTS WITH MAN

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INTRODUCTION

The nutrition of the human body consists mainly in the transformation of food into body material and the ultimate transformation of the energy potential in both food and body material into such forms of energy as heat and muscular work. The transformations of both food and body material occur largely in accordance with the needs of the body for energy. To understand the laws governing the nutrition of the body, knowledge regarding these transformations of matter and energy is essential.

To obtain such knowledge it is necessary to have some means of determining the intake and output of both matter and energy by the body. This involves the use of some form of apparatus that will give an accurate measurement of the gaseous exchange and the energy production of the body. Such an apparatus is the so-called respiration calorimeter employed in connection with the nutrition investigations of the Department of Agriculture.

The first apparatus of this kind constructed in this country was developed in connection with these investigations. Work on this device was begun in 1892 by Prof. W. O. Atwater at Wesleyan University, Middletown, Conn. When the Department of Agriculture undertook an inquiry into the food and nutrition of man in 1894 as a logical outgrowth of the earlier work of Prof. Atwater for the Smithsonian Institution and the United States Department of Labor, the need of some means of determining the income and outgo of matter and energy in the body was recognized, and the general plan of work to be undertaken as part of the inquiry was made to include experiments with the respiration calorimeter which had been devised for measuring factors of outgo.

For use in the study of the output of matter by the body, the device was similar in principle to the respiration apparatus of Pettenkofer(16),[1]

[1] Reference is made by number to "Literature cited," p. 346–347.

with alteration in detail in accordance with modification in methods of investigation, but in its equipment for the measurement of the output of heat it was quite original. Prof. E. B. Rosa, then of Wesleyan University and associated with Prof. Atwater in the investigations, devised a method of preventing the passage of heat through the walls of the respiration chamber, and provided for carrying out and measuring the heat generated within it. The term "respiration calorimeter" was applied to the Atwater-Rosa device to indicate that it performed simultaneously the functions of both a respiration apparatus and a calorimeter.

Experiments with the respiration calorimeter have been continued as part of the nutrition investigations of the Department of Agriculture during the 20 years or more since they were begun. With the progress of the work many modifications have been introduced for the purpose of making the apparatus simpler, easier, and more economical to operate than the original, while yielding more complete and more accurate data. Descriptions of the apparatus in its original form and its later modifications, and the results of a large number of experiments with it, have appeared in former publications of the Department (1, 2, 3, 4, 6, 9) and have become a part of the data commonly included in textbooks and works of reference.

As a result of the work of Atwater and his associates, the investigator has been provided with an apparatus of precision and a method of investigation which, with adaptation in different laboratories to meet varied experimental conditions, have proved valuable for a range of work even wider than was originally anticipated. In the nutrition laboratories of the Department of Agriculture it has been employed in the form described in the present publication in studies of the utilization of food and the performance of muscular work, and a recent development, to be described in detail in a later publication, has been adapted to studies of problems in plant physiology. At the Institute of Animal Nutrition, State College, Pa., Dr. H. P. Armsby employs a respiration calorimeter, which he has adapted from the original Atwater-Rosa type of apparatus, in investigations of the nutrition of farm animals conducted in cooperation with the Department of Agriculture. In other inquiries besides those of the Department respiration calorimeters have proved of great value in investigations of different but related character. Investigators have modified and improved the original form to suit their special needs, though this method of research has long passed the experimental initial stage and has become recognized as possessing great possibilities where accurate measurements of energy values and gaseous exchange are needed to supplement the data which the investigator secures by other methods.

The respiration calorimeter employed at the present time in the nutrition investigations of the Department of Agriculture is a development of

the one used for over 12 years in the laboratory of Prof. Atwater. In 1907, when because of illness he discontinued his connection with the research, the respiration calorimeter was transferred to Washington. To move the apparatus it was necessary to dismantle it completely, so that to set it up again in the laboratory provided for it in the new building of the Department involved its practical reconstruction. Advantage was taken of the opportunity thus afforded to modify it in many important details, with special consideration for simplicity of structure and convenience of operation. The reconstructed apparatus has been briefly described in a former publication of the Department (15) and elsewhere (14). The experience with this apparatus has suggested further improvements that have been incorporated from time to time, with the result that the work of conducting an experiment with the respiration calorimeter is much less than formerly, and a degree of accuracy of measurement is obtained that was not possible with the apparatus in its earlier state. The present publication describes this greatly improved respiration calorimeter in detail. A general view of the apparatus is shown in Plate XXX.

PRINCIPLE OF THE RESPIRATION CALORIMETER

The principle of the respiration calorimeter now in use in the nutrition investigations is the same as that of the later form of the apparatus employed in the investigations formerly conducted at Wesleyan University. For the determination of gaseous exchange the device is similar to the respiration apparatus of Regnault and Reiset (17), having a respiration chamber and a system of air-purifying devices connected in series in a closed circuit. The air confined in the circuit is kept in circulation, the respiratory products imparted to it by the subject in the chamber being constantly removed and oxygen constantly supplied to replace that used by the subject. For the determination of heat produced in the chamber the device is a constant-temperature, continuous-flow, water calorimeter, in which the calorimetric features of the original Atwater-Rosa apparatus are retained. These provide for preventing the passage of heat through the walls of the chamber and for taking up the heat by a current of cold water as fast as it is generated in the chamber. The determination of respiratory exchange and energy transformation, to be of value, demands a high degree of accuracy in the fundamental measurements, and it follows that the instrument with which they are made must be precise and finely adjusted, sensitive to slight changes within, and protected from the effects of fluctuations occurring outside of it.

Of fundamental importance in the device is a chamber with walls that are air-tight and heatproof. It must be so large that the subject may live in it in comfort during the time of an experiment, which may continue several hours or several days, and yet not so large that its volume

will prevent the accurate measurement of the amounts of the different gases in the air inclosed. Its walls must be absolutely air-tight, because any leakage of air would nullify the determination of the respiratory exchange, and there must be no passage of heat through them, because any transference of unmeasured heat into or out of the chamber would introduce error into the determination of the amount of energy produced within it. In the following pages the construction of the chamber of the apparatus is described, and the auxiliary apparatus and methods employed in determining the respiratory exchange and energy production of a subject in the chamber are explained in detail.

CONSTRUCTION OF THE RESPIRATION CHAMBER

The respiration chamber is approximately 1.96 meters long, 1.96 meters high, and 1.19 meters wide, the total volume of the empty chamber being close to 4,570 liters. On the side walls are hooks for clothing and shelves for books, food receptacles, and the like. The furniture consists of a chair and a table, and a cot is provided in experiments lasting a day or more. These may be folded into small bulk when not in use, to provide as much space as possible in which the subject may move about, if the nature of the experiment allows freedom of muscular movement. In experiments of several hours' duration, when the subject is to be very quiet, the ordinary chair and the cot are replaced by an adjustable reclining chair in which he may sit or recline at will, the change in position involving almost no effort. When the experiment involves the performance of muscular work, an ergometer of special construction for measuring the amount of muscular work done is included. There is a telephone for communication between the subject inside the chamber and the observer on the outside. Every provision is made for the convenience of the subject within the limits of the experimental conditions. (See Pl. XXXV, fig. 1.)

In one wall of the chamber, facing a window of the laboratory, there is an opening about 48 cm. wide by 54 cm. high, through which the subject enters and leaves the chamber (Pl. XXX). During an experiment this is closed with plate glass sealed in place, and thus serves as a window. On bright days this window will admit sufficient light for reading or writing, but further light is generally provided by a small electric lamp inside, which the subject may locate according to his desire. Near the center of one end of the chamber is a smaller opening through the walls, called the "food aperture," which is closed by a tube having a valve or trap on one end opening into the chamber, and another on the other end opening to the exterior. This comprises an air lock, through which articles such as food receptacles, books, etc., may be passed into or out of the chamber without any interchange of air between the interior and the exterior of the chamber other than that due to displacement by the articles placed in the

aperture. Several small openings in the walls provide for the passage of air pipes, water pipes, and wires for electric current (Pl. XXXII, fig. 1).

The walls, ceiling, and floor of the chamber are of 16-ounce copper, tinned on both sides. Large sheets of copper are used, so that there will be few joints in the walls. The sheets are joined with tightly locked seams heavily soldered, making them air-tight. When the soldering was completed, the tightness of the walls was tested by air pressure, the level of the column of water in a manometer connected with the chamber being observed at frequent intervals for several hours. It remained constant, due allowance being made for the effect of change of temperature or barometric pressure during the test.

The copper-walled chamber is attached to the inside of a framework of structural iron (Pl. XXXI, fig. 2). The sills and ceiling plates are angle iron with legs about 63 by 63 mm., and are bolted together at the corners. The studding for the side walls and the joists for the floor and the ceiling are of light-weight channel iron about 63 mm. wide, bolted to the plates with stiff angles or elbows, with the width of the channel at right angles to the length of the plates (Pl. XXXI, fig. 1.) The chamber is attached to the framework by long, slender stove bolts passed through holes in the edge of the channels and screwed into brass nuts soldered to the outer surface of the copper. Between each channel and the copper attached to it is a strip of wood about 6 mm. thick and 3.5 cm. wide, to prevent actual metallic contact and to interfere with the transference of heat from the copper wall to its iron supporting structure. Between the copper floor and the floor joists is a layer of asbestos lumber about 9 mm. thick (shown in Pl. XXXI, fig. 1), to provide a solid support for the thin metal floor of the chamber.

To the outer edge of the iron structure is attached a surface of sheet zinc corresponding to the copper wall, ceiling, and floor of the chamber (Pl. XXXII, fig. 1). Sheet zinc about the same weight as that of the copper was used. Washers slipped under the heads of the bolts by which the copper wall is attached serve to bind the zinc to the iron. The chamber is thus provided with double metal walls separated by a dead-air space about 7 cm. across, the purpose of which is explained on page 331, in the description of the method of preventing the passage of heat through the walls of the chamber.

The framework of the chamber was made of structural iron, to secure rigidity and to provide a strong support for any apparatus that it might be found advantageous to employ in experiments in which muscular work would be performed. It entails, however, an undue amount of care in making the calorimetric measurements to avoid error that might result because of the heat capacity and thermal conductivity of the iron, as explained on page 338. Should opportunity to reconstruct the apparatus arise, the iron would be replaced by some material that would provide ample rigidity and strength of structure and have less thermal capacity and conductivity.

The chamber does not rest upon the floor of the laboratory, but is supported about 45 cm. above it by a structure of channel iron (Pl. XXXII, fig. 1), with upright pieces 10 cm. wide, which rest on floor plates and are bolted to the ceiling and between which are cross pieces 7.5 cm. wide, on the lower of which rests the chamber. To this structure is also attached the framework for supporting an outer covering of cork board, described on page 334. This covering is constructed so that it may be easily detached to provide ready access to any part of the zinc wall. The outer surface of the cork board is covered with a layer of museum board 6 mm. thick, painted white on the outside (Pl. XXX).

DETERMINATION OF RESPIRATORY EXCHANGE IN THE CHAMBER

The atmosphere of the empty chamber contains oxygen, nitrogen, water vapor, and carbon dioxid in proportions like those of ordinary air. When the subject enters the chamber, the proportions begin to change, with the consumption of oxygen and the elimination of water vapor and carbon dioxid. The removal of the water vapor and carbon dioxid from the air and the restoration of oxygen to it in such manner that the quantity of each may be accurately measured form the basis of the determination of the respiratory exchange in the chamber.

The respiratory products are constantly carried out of the chamber by a current of air that is kept in circulation through the system. The air leaves the chamber in a pipe which opens near the floor at one end, passes through purifying devices, and returns to the chamber in a pipe which opens near the ceiling at the other end. The purifying devices, called "absorbers," remove from the air passing through them the water vapor and carbon dioxid imparted to it by the subject. The increase in the weights of the absorbers in a given period shows the quantities of water vapor and carbon dioxid carried out of the chamber during the period. In addition to the data thus obtained, account must be taken of changes in the quantities of water vapor and carbon dioxid in the air of the chamber, as shown by analyses of samples of the air at the beginning and the end of the period, in determining the quantities produced in the chamber during the period (p. 310).

Oxygen is supplied to the chamber from a cylinder of the gas under pressure, and the loss in weight of the cylinder shows the quantity admitted during the period. To determine from data thus obtained the quantity of oxygen consumed by the subject, allowance must be made for changes in the quantity of oxygen in the air of the chamber.

AIR-TENSION EQUALIZER

The volume of air in the chamber varies constantly with the admission of oxygen and the removal of water vapor and carbon dioxid, and also with changes in the temperature of the air in the chamber and in the barometric pressure of that outside. This might result in undesirable

variations in the pressure of the air in the chamber unless provision were made for corresponding fluctuations in the capacity of the system. This is accomplished by attaching a flexible diaphragm of thin rubber or a sensitive spirometer to a small tube opening into the chamber, which serves as a tension equalizer, keeping the air of the chamber always at the barometric pressure of that of the laboratory (Pl. XXX).

AIR-PURIFYING SYSTEM

The circulation of air is maintained by a rotary air pump, which has a capacity of close to one-fourth of a liter per revolution and is driven at a rate of about 250 revolutions per minute, so that the air is forced through the purifying system at a rate of 60 to 70 liters per minute. An electric motor of one-eighth horsepower is sufficient to run the pump and to move the air through the absorbers (Pl. XXXII, fig. 2).

All piping in the air-circulating system is brass pipe of the so-called half-inch size, which has an internal diameter of 15 mm. The apertures of the air passages in the purifying devices are also of this size. This has been found sufficient to conduct the air at the desired rate without undue resistance, the pressure in the section of pipe between the compressor and the first water absorber, where it is higher than in any other part of the system, being less than 40 mm. of mercury.

The motor, the rotary air pump, and the absorbers for water vapor and carbon dioxid are assembled on a suitable-sized stand or table, with three shelves, called the "absorber table" (Pl. XXXIV, fig. 1). The motor and pump are on the lower shelf, and on the middle shelf are the purifying devices in a series or train; first, the absorbers for water vapor, and next, the absorbers for carbon dioxid. The air pipe from the respiration chamber passes to the pump and then to the inlet end of the absorber train. From the outlet end of the train the air pipe returns to the chamber, the ingoing and outgoing pipe passing through the walls in two apertures close together. Inside the walls the pipes extend to opposite ends of the chamber, the end of the ingoing pipe being near the top of the chamber, and that of the outgoing pipe near the bottom.

Two absorber trains are set up in parallel and are used in alternate periods, the air pipe at each end of the trains being branched for this purpose. There is a valve in the piping at each end of each train, and the change from one train to the other involves merely closing the valves for one train and opening those for the other. When ordinary wheel valves are used, as shown in the illustration, the motor is stopped for the few seconds necessary to make the change; but the valves at each end of the purifying system may be replaced by a suitable 3-way cock or air trap at the point where the air line branches at each end of the train, and the two cocks may be actuated by the same shaft, so that the air current can be shunted from one train to the other with a single motion from either end of the absorber table and while the air pump is

still running. By actuating the shaft electrically the change can be made by the observer at a distance, or a clock can be used to close the electric circuit at any given time and thus make the change automatically.

While the air is passing through one train the other is disconnected, the absorbers weighed, the absorbent renewed if necessary, and the train again connected in position. The absorbers are joined together by couplings which are attached to the inlet and outlet tubes by stout, flexible rubber tubing. Rubber washers between the halves of each coupling make a tight joint. A similar coupling connects each end of the train with the air pipe. When the whole train is in position it is tested for tightness, with the air in the system at a pressure of about 1 meter of water, which is considerably more than the highest pressure in any part of the train in service.

Removing Water Vapor from the Air

In the purifying system the air passes first through sulphuric acid, which removes all water vapor from it. The acid container, which is in effect a modified gas-washing bottle of moderately large capacity (Pl. XXXIII, fig. 1), was devised in connection with these investigations. A strong glass bottle about 2½ liters in capacity (about 24 cm. in height and 12 cm. in diameter), with a wide mouth, is fitted with a special ground-glass stopper, in the top of which are sealed an entrance and an exit tube, each 15 mm. in internal diameter. The entrance tube, which is in the middle of the stopper, extends to very near the bottom of the bottle, and terminates in a bulb about 4.5 cm. in diameter, which has several holes about 4 mm. in diameter in the sides and bottom, the total area of the holes being about equal to that of the cross section of the tube. Surrounding the bulb is a bell of about 7.5 cm. diameter, attached to the tube at a point a little above that at which the bulb is attached. The bell is completely open at the bottom, and has a row of holes about 7 mm. in diameter around the side at a level just above the top of the bulb.

When charged, the bottle is filled with acid to a level a little above the row of holes in the bell, about 750 c. c. of acid being sufficient for this purpose. The air escaping through the holes in the bulb and in the bell is broken into bubbles, which in passing through the acid are deprived of moisture. The passage of the air through the acid keeps it vigorously stirred, acid coming up through the bottom of the bell to replace that forced out through the holes at the sides. To prevent globules of acid from being spattered or carried by the air into the exit tube, the bottom of the stopper, which is about 6 cm. below the top, is nearly closed, an annular space about 8 mm. across being left around the tube that projects to the bottom of the bottle to provide for the exit of air. Into the space thus formed in the interior of the stopper are placed lumps of

pumice stone, which effectually prevent visible particles of acid from being spattered into the exit tube or carried into it by the air current.

During several years' use these bottles have proved to be very satisfactory. Before they were used in experiments a large number of tests of their efficiency were made, in which air was passed at various rates up to 80 liters per minute through three of the bottles in series, the first one containing water, in which the air became very moist, and the other two charged with acid. It was found that the moist air leaving the first bottle could be passed through the acid in the second bottle until it was diluted to nearly twice its bulk before the third bottle increased appreciably in weight. No gain in weight was ever observed in a third acid bottle included in the series in some of the tests. In many of these tests the water vapor in the air leaving the water bottle was very nearly saturated at the temperature of the laboratory. These conditions imposed as severe a test on the capacity of the device to remove all moisture from the air flowing through it as any that would occur in respiration experiments.

In practice, two bottles are used in series and the first one is recharged when the acid in it has become diluted to a volume indicated by a mark on the bottle, in which case 750 c. c. of acid have usually absorbed 500 to 600 c. c. of water. Each bottle with its charge of acid weighs not far from 2,600 gm. The two acid bottles will stand side by side on the pan of the large sensitive balance, and are weighed together to an accuracy of 0.1 gm. The increase in the weight of these two absorbers in a given period shows how much water vapor has been carried out of the chamber during the period.

Removing Carbon Dioxid from the Air

The air from the acid bottles passes next through bottles containing soda lime (a mixture of caustic soda and quicklime), which deprives it of carbon dioxid. The soda-lime container that has been in use for several years consists of an ordinary wide-mouth bottle about 25 cm. in height and 13 cm. in diameter. The mouth of the bottle is closed with a No. 12 rubber stopper, through which pass an inlet tube and an outlet tube of brass pipe, with a bore of 15 mm. The inlet tube extends nearly to the bottom of the bottle. The lower opening of this tube is protected with brass wire gauze to prevent particles of soda lime from entering it. The outlet tube extends outward from the under side of the stopper. When the stopper is tightly sealed and bound in place, soda lime in particles about the size of a dried pea or smaller is introduced through the outlet tube until the bottle is filled quite near to the top. Each bottle when thus charged contains a little over 2 kgm. of soda lime and weighs about 4 kgm.

Two of these bottles are used in series, and each one is kept in use until the appearance of the soda lime indicates that it is no longer effi-

cient enough for further use, which is shown by its change in color. The fresh, somewhat moist soda lime is a dingy white, but in use it becomes much lighter and clearer, owing to both the absorption of carbon dioxid and the loss of moisture, which is taken from the soda lime by the dry air. The bottle may be recharged whenever all of the visible surface of soda lime has thus changed, though if the whitened material has not become compacted into a hard mass which will prevent air from passing through it the efficiency of the soda lime may be restored by passing air containing water vapor through the bottle until the dry material has absorbed about as much moisture as it contained originally, as may be judged from the darkening of the color. In this manner a given charge may be used at least twice. In either case, if the bottle is opened, any soda lime not compacted but still remaining granular may be used again, especially if it is mixed with a large proportion of fresh material. In an ordinary rest experiment in which carbon dioxid is removed from the air current at a rate of 25 to 30 gm. an hour, the material in one of these bottles will absorb at least 150 to 200 gm. of carbon dioxid before all the soda lime has whitened.

These bottles are quite satisfactory in many respects, but in using them great care is necessary to avoid leakage of air between the stopper and the neck of the bottle, or between the stopper and the tubes passing through it, especially after the bottle has been in use a short time. When these joints are made, they are thoroughly painted with shellac, but since the stopper is quite flexible there is possibility of breaking the coating in using the bottle. Some of these chances for leakage will be eliminated by a special cover designed to be clamped to the top of the bottle, into which the inlet and outlet tubes are soldered.

The soda lime is used moist rather than dry because it is more efficient in that condition. In passing through this moist material the dry air from the water-vapor absorber takes moisture from it. The air from the carbon-dioxid absorber is therefore passed through another bottle of sulphuric acid, to catch the moisture given off by the soda lime. This bottle is weighed with the two soda-lime bottles to find the amount of carbon dioxid removed from the air current coming from the respiration chamber, the three bottles standing together on the pan of the large balance being weighed as a unit. Their total weight, which is less than 12 kgm., is ascertained accurately to 0.1 gm.

Trap for Atomized Sulphuric Acid

Though the pumice in the stopper of the sulphuric-acid bottle effectively arrests visible particles spattered up by the vigorous agitation of the acid or blown up in the air current, acid in some condition, apparently resembling vaporous exhalation, escapes in the air leaving the bottle. The amount of acid that leaves the absorber is so small that even after the air

has been passing for several hours the loss has no effect on the weight of the absorber within the limits to which the weight is determined; yet if the acid carried in this manner from the bottle mentioned in the preceding paragraph is allowed to escape into the air of the chamber, it has a noticeable effect upon the respiration of the subject in a few minutes. To avoid this effect, the air from the absorber passes through a trap which removes the acid spray before it enters the pipe for air returning to the chamber. For several years the trap consisted of sodium carbonate between two layers of cotton wool inclosed in a metal cylinder about 15 cm. long and about twice the diameter of the air pipe. Later, a piece of heavy glass tubing was substituted for the metal cylinder (Pl. XXXIV, fig. 1), and it was observed that the air was freed from acid apparently by mechanical filtering rather than by chemical action between the acid and the carbonate. The first layer of cotton arrested all the acid that reached the trap during several months' use, and the carbonate appeared to be unnecessary. In accordance with this suppositoin, the cotton and carbonate in the trap were replaced by pumice stone in pieces very much smaller than those in the stopper of the absorber, and this has prevented the passage of the acid spray into the pipe for ingoing air.

Supplying Oxygen to the Air

Oxygen to replace that used by the subject is admitted directly to the chamber through a copper pipe of a bore of about 5 mm. passing through an opening in one wall. The supply of oxygen is contained under pressure in a steel cylinder, the outlet of which is closed with a pressure-regulating valve by which the rate of admission of oxygen is governed. No attempt is made to keep any definite proportion of oxygen in the air. The regulator valve is usually set to admit oxygen at a rate that will keep the volume of gas in the chamber fairly constant, as indicated by the rubber diaphragm or the spirometer serving as an air-tension equalizer for the chamber. The valve may be opened or closed by hand as regulation of the volume is necessary; or by causing the diaphragm or spirometer when nearly full to open and when nearly empty to close an electric circuit, an auxiliary valve may be operated so that the admission of oxygen is automatically regulated to keep the total volume of air in the chamber within the desired limits. A simple auxiliary valve consists of a pinch-cock actuated by an electromagnet so as to compress or release the rubber tubing connecting the outlet of the regulating valve with the end of the pipe taking oxygen to the chamber.

The steel cylinder containing the oxygen is suspended from one arm of a large sensitive balance, and from the other arm is suspended a similar cylinder, empty, to serve as a counterpoise (Pl. XXXIV, fig. 1). The loss in weight of the charged cylinder in a given period shows the amount of gas admitted to the chamber during the period. Though each cylinder

weighs nearly 60 kgm., the loss in weight is ascertained to an accuracy of 0.1 gm.—that is, the volume of gas supplied, which may reach 80 liters or more per hour, may be determined within 100 c. c.

This method of determining the quantity of gas admitted to the chamber is very precise, but it involves time and effort that could be saved by the use of a gas meter if the mere reading of the dial of the meter would show the quantity with equal precision. In a number of experiments the gas from the weighed cylinder was passed through a calibrated test meter before it entered the chamber, to determine whether the volume of gas admitted could be ascertained in this manner with sufficient accuracy. It was found that when the gas was admitted at a fairly uniform rate throughout the period, the volume as determined from the meter reading would agree quite closely with that computed from the loss in weight of the cylinder; but when it was necessary at times to admit gas rapidly, the agreement was not so close, a correction being necessary for increase of pressure in the meter. The time and labor involved in reading, recording, and correcting for increased pressure in the meter are at least as much as those of weighing the cylinder.

In most of the investigations with this respiration calorimeter the gas contained in the cylinder, and consequently that admitted to the chamber, was about 97 per cent oxygen. It was derived from liquid air and was virtually free from carbon dioxid and water, but contained a small proportion (about 0.3 per cent) of nitrogen and an appreciable proportion (about 2.7 per cent) of argon, for which allowance must be made in computing from the loss in weight of the cylinder the quantity of oxygen admitted to the chamber. In making the correction it is sufficiently accurate to consider the impurity as all argon. It is possible, however, to obtain oxygen that is so nearly free from other gases that the error involved in disregarding them is inconsiderable.

DETERMINATIONS OF THE AMOUNTS OF RESIDUAL GASES

As has been stated (p. 304), to determine the amount of oxygen consumed and of carbon dioxid and water vapor produced by the subject in the chamber during a given period, allowance must be made for any changes that have occurred in the composition of the air of the chamber—that is, in the quantities of different gases residual in the chamber. These are ascertained from analyses of samples taken at the beginning and the end of the period. Because of convenience, the samples are taken, not directly from the air of the chamber but from that passing through the air pipes outside of the chamber. It is assumed that the air in the outgoing pipe has the same composition as that in the respiration chamber. Though the composition of the latter is constantly changing, an electric fan keeps the total mass of air in the chamber energetically stirred to prevent stratification and to mix the varying component gases as thoroughly as possible. It seems probable, there-

fore, that the composition of the air in the outgoing pipe fluctuates quite uniformly with that of the total air in the chamber.

Analysis of Sample for Water Vapor and Carbon Dioxid

For the determination of the amounts of moisture and carbon dioxid residual in the chamber at the end of each period, a portion of the air coming from the chamber at that time is shunted from the main current through a petcock in the air pipe at a point between the rotary pump and the first sulphuric-acid bottle, and is passed first through a small purifying system and then through an accurate gas meter, which rests on the top shelf of the table for the large absorbers, as seen in Plate XXXVI, figure 2. The air leaving the meter is passed through sulphuric acid to remove the water vapor taken up by it in passing through the meter, and is then returned to the main current flowing from the large absorbers to the chamber. The water-vapor absorbers of the small train are specially devised, somewhat resembling those of the large train, but of such size that they may be weighed on an analytical balance (Pl. XXXIII, fig. 2). A 4-inch U tube with side outlets and well-ground glass stoppers makes a serviceable soda-lime container. A train consisting of one acid bottle, one U tube, and another acid bottle very efficiently removes all water vapor and carbon dioxid from the air passing through it at a rate of about 3 liters per minute.

The small absorbers are weighed on an analytical balance to an accuracy of 0.1 mgm., each unit, when charged, weighing less than 100 gms. The increase in the weights of the units shows the quantities of water vapor and carbon dioxid in a given volume of the air. Usually 10 or 20 liters of air, as indicated by the meter, are passed through the train, the actual volume being ascertained by correcting the meter reading, when necessary, for the calibration of the meter and for the temperature and barometric pressure of the air passing through it.

Analysis of Sample for Oxygen

For the determination of the proportion of oxygen in the residual air a small sample, about ½ liter, is taken from the returning air in the pipe between the large purifying system and the respiration chamber, where it is free from water vapor and carbon dioxid. In Plate XXXVI, figure 2, a rubber bag for holding the sample is seen hanging from an outlet in the air pipe at the end of the absorber table. A modified Haldane burette is used in the determination, the oxygen being absorbed by a potassium-pyrogallate solution in a Hempel pipette.

Computation of Volumes of Gases Present

The actual determination of the proportion of oxygen in the air is not necessary at the end of each period. The volume of oxygen present in the air of the chamber may be computed by subtracting from the actual

volume of total air present the sum of the volumes of carbon dioxid and water vapor present, as shown by analyses of the residual air, and the volume of nitrogen, including that present at the beginning of the period and that added with the oxygen admitted during the period, due allowance being made in the latter for any impurity.

To compute the total quantities of carbon dioxid and water vapor in the air of the chamber, the volumes corresponding to the weights of the gases removed by the small absorber system from the air sample measured by the meter are multiplied by a factor representing the ratio between the volume of the sample and the total volume of air in the chamber when both are reduced to standard conditions of temperature (0° C.) and of pressure (760 mm. of mercury). The necessity for accuracy in the analysis of the sample is shown by the fact that under usual experimental conditions there are more than 4,000 liters of air in the chamber; hence, any error in the determination of the quantities of water vapor and carbon dioxid in a 10-liter sample is multiplied over 400 times.

The actual volume of air in the chamber under standard conditions depends upon the capacity of the chamber and the barometric pressure and temperature of the air in it. These factors must be accurately determined, since a difference of 1 mm. in the pressure means a difference of over 5 liters in the computation of the actual volume of gas, while a difference of 1 degree in the temperature means a difference of about 15 liters in the total volume. An error in these determinations has some effect upon the computation of the quantities of residual gases, though the effect of any error likely to occur upon the quantity of water vapor would be quite insignificant, as there are seldom more than 90 liters present, and commonly less. The effect on the computation of carbon dioxid would be somewhat larger, as there might be in some circumstances 100 liters or more in the air; but under ordinary conditions the quantity is decidedly less, and the error would be relatively unimportant. The effect would be greatest upon the computation of the quantity of oxygen, as under normal conditions there could be as much as 850 liters present.

MEASUREMENT OF CAPACITY OF THE CHAMBER

The capacity of the chamber is known very accurately. It may be computed from the dimensions of the chamber, and it may be directly ascertained by determining the proportion of oxygen in the well-stirred air of the sealed chamber before and after the admission of a known volume of the gas.

MEASUREMENT OF BAROMETRIC PRESSURE OF THE AIR

The barometric pressure of the air of the chamber, which, because of the air-tension equalizer mentioned on page 304, fluctuates the same as

that of the laboratory, is determined by means of an accurate barometer mounted on the walls of the laboratory. The height of the mercury column in the barometer tube may be read by a vernier to 0.01 mm. The barometer has been standardized by the Weather Bureau.

MEASUREMENT OF TEMPERATURE OF THE AIR

The temperature of the total mass of air in the chamber is not so easily determined as its pressure. Even when the walls of the chamber are at uniform temperature and no heat is generated in it, the temperature of the air may not be uniform in all parts of the space. When heat is being generated in the chamber and is being absorbed and removed as fast as it is generated, so as to maintain constancy in what is assumed to be the average temperature, there is a considerable difference between the temperature and the consequent density of the air near the source of heat and that of air near the heat absorber. It seems reasonable to suppose, however, that with the tendency of warm air to rise and of cold air to fall, and particularly with the vigorous agitation of the air of the chamber by the electric fan, the warmer and colder volumes of air will be very rapidly mixed, and more or less complete uniformity of temperature quickly established throughout the whole mass of air.

The temperature of the air of the chamber is measured by means of an electric-resistance thermometer. The method of measurement employed is based upon the fact that the resistance of a wire of pure metal to an electric current changes definitely with a change in its temperature and also that the resistance of the wire, and particularly its change in resistance, whether large or small, due to corresponding changes in temperature, may be measured with extreme accuracy by means of a suitable Wheatstone bridge. The device used in the respiration calorimeter comprises specially mounted bare nickel resistance wire in the chamber, connected with a special Wheatstone bridge, called the "temperature indicator," on the observer's table (Pl. XXXVI, fig. 1).

The nickel wire, the resistance of which varies with changes in the temperature of the air of the chamber, is in six coils of equal resistance, each of which is mounted in a rectangular wooden frame about 10 by 13 cm. that is suspended in the air about 4 cm. from the wall of the chamber, on supports attached to the wall. The wire is stretched across the space in the frame between two slender wooden rods about 5 cm. apart, with successive strands of the coil about 5 mm. apart. Since very little of the wire is in contact with the support, it is but little, if at all, affected by the temperature of the frame, the object of the construction being to eliminate lag in the action of the thermometer. The exposed wire very rapidly acquires the temperature of the air of the chamber, and hence responds instantly to any changes in it. The six coils are distributed on the walls and ceiling in different vertical and horizontal positions, to

integrate different temperatures if there are differences, and as the air is very thoroughly stirred by the electric fan previously mentioned, it is probable that the resistance thermometer shows the average temperature of the air of the chamber. In the interior view in Plate XXXV, figure 1, two of the frames are plainly shown with a wide-mesh wire screen before the resistance wire to protect it against contact with any object that would cause a short circuit between two parts of the wire, as well as against injury.

The six coils are connected in series by well-insulated No. 16 copper wire, and similar wire leads from the terminals of the series, through a rubber stopper in a small opening in one wall of the chamber, to a special switch on the observer's table, by which they may be connected in one arm of the Wheatstone bridge. The purpose of the switch is to provide means for using with these coils the same bridge that is used with other coils for measuring the temperature of the walls of the chamber and that of the body of the subject, as explained later in this paper. This switch must be designed to avoid the error that would result from introducing appreciable resistance of the switch contacts into the bridge circuits. The connections between the bridge and the resistance coils include a compensating lead to eliminate from the measurement of the resistance of the coils the effect of both the resistance of the leads and any change in their resistance due to change of temperature. The contact that is moved along the slide wire of the bridge, to restore balance when the resistance of the thermometer coils has changed, is in series with the battery, so that contact resistance introduces no error in the measurement.

The six coils have a total resistance of about 20 ohms at 20° C. Since the resistance of nickel wire varies approximately 0.4 per cent per degree at the usual temperatures of the experiments, their total change in resistance would be close to 0.08 ohm for a change of 1° in the temperature of the air of the chamber. The resistance of the slide wire of the Wheatstone bridge will balance the bridge circuit for the change of resistance in the coils that would result from a change of 5° in the temperature. By means of several coils of manganin wire, which may be connected in series with the slide wire, the total range of the bridge may be extended, but under usual experimental conditions the temperature of the air is allowed to change as little as possible. Whether the change is large or small, it must be measured accurately. A change of resistance in the thermometer coils resulting from a change of 0.01° in the temperature of the air will upset the balance of the bridge sufficiently to cause a deflection of the sensitive reflecting D'Arsonval galvanometer that indicates when the bridge is balanced. The balancing point of contact may be moved along the wire a distance sufficiently small to restore the balance, and the scale of the slide wire will indicate the distance.

OBSERVER'S TABLE

The Wheatstone bridge described above and the telephone mentioned on page 302 are located on the table beside the chamber (Pl. XXXVI, fig. 1) at which the observer sits while controlling the apparatus. The same bridge is employed in the determination of other temperatures, as described beyond. Other devices on the table serve to indicate and regulate temperature conditions inside and outside the chamber, as explained in detail in the sections which follow.

DETERMINATION OF THE QUANTITY OF HEAT PRODUCED IN THE CHAMBER

Energy expended by the human body for any purpose, such as the performance of muscular work, the maintenance of body temperature, or whatever, results in the production of heat, which is eventually dissipated from the body; hence, the measurement of the quantity of heat dissipated by the body under given conditions affords data for the determination of the quantity of energy expended. Heat escapes from the body in two ways: As latent heat of water vaporized from the lungs and skin and as sensible heat, by conduction, convection, and radiation from the surface of the body to the air and to objects in the chamber. Both latent heat and sensible heat are carried out of the chamber and measured.

MEASUREMENT OF LATENT HEAT

The water vaporized by the lungs and skin leaves the chamber in the outgoing air, unless it is precipitated by contact with some object in the chamber whose temperature is below the dew point for the conditions prevailing, but the temperature of the air and of objects in the chamber is controlled so that precipitation is not likely to occur. The quantity of heat leaving the chamber as latent heat of water vapor in any given period is determined by multiplying the weight of the water vapor absorbed from the outgoing air during the period by the factor 0.586, which according to determinations made by Smith (18), represents the number of Calories of heat required to vaporize a gram of water at 20° C. All measurements of heat with the calorimeter are expressed in terms of Calories at 20° C., 1 Calorie being taken as the amount of heat required to raise the temperature of 1 kgm. of water 1° C.—i. e., from 19.5° to 20.5°, the specific heat of water being taken as unity at 20° C. The determinations by Smith were made in accordance with the conclusion by Barnes (7) that the mean small calorie is equivalent to 4.1877 international joules. Dickinson, Harper, and Osborne (10), in work on the latent heat of fusion of ice, assumed 4.187 international joules equal to 1 small calorie at 15°, in which case 4.183 joules would be equivalent to 1 small calorie at 20° C. The latter value is used in these investigations (p. 342), but the difference between this and the value by Barnes has no significant effect upon the factor for latent heat here employed.

MEASUREMENT OF SENSIBLE HEAT

The energy eliminated from the body as sensible heat, which is much greater in amount than that latent in water vaporized from the body, is practically all carried out in a current of water which circulates in the chamber through a device called the "heat absorber," though a small quantity of it may become latent in water vaporized from objects in the chamber, in which case it may leave the chamber as latent heat of water vapor in the outgoing air. If the weight of the water that flows through the absorber during a given period, as stated in kilograms, is multiplied by the difference between the temperature of the water as it enters and that as it leaves the absorber, as measured in degrees centigrade, the product will show the quantity of heat removed as expressed in Calories, at the mean temperature of the water flowing in the absorber. These are converted into Calories at 20° by making due allowance for the specific heat of water at the mean temperature of the flow as compared with that at 20° (4, p. 56; 19, p. 229).

The rate at which heat is removed from the chamber is regulated to prevent fluctuations in the temperature of the air of the chamber, which falls when the rate is too fast and rises when it is too slow. To avoid chance for error in the determination of the volume of air in the chamber, which depends upon the accuracy of the measurement of its temperature (p. 313), and to some extent also for the comfort of the subject, it is desirable to keep the temperature of the air as constant as possible. The temperature to be maintained depends upon the nature of the experiment, but it is commonly not far from 20° C. Whatever the requirement may be, by proper control of the temperature at which the water enters the heat absorber, and of the rate at which it passes through the absorber, the removal of heat from the chamber may be made to accord with its production within it to such an extent that the temperature of the air of the chamber may be kept constant within narrow limits. The most convenient practice is to maintain a constant rate of flow and to vary the temperature of the water entering the heat absorber according to the amount of heat to be absorbed.

Heat Absorber

The heat absorber, which is suspended near the ceiling of the chamber, about 10 cm. from the sides, consists of brass pipe of 7 mm. internal diameter (so-called ⅛-inch pipe), along which disks of sheet copper 5 cm. in diameter are soldered 3 mm. apart to increase the area of the heat-absorbing surface. The total length of pipe in the absorber is not far from 11 meters, and there are more than 2,500 disks on it, so that several square meters of surface are exposed to the air of the chamber. Though the total quantity of water in the absorber is not over 400 c. c., it is possible, by control of the temperature and rate of flow of the water, to vary the rate of removal of heat from the chamber within wide limits.

The coil passes once around the chamber and back again, the two pipes lying not quite 5 cm. apart, with the disks on one slightly overlapping those on the other. The purpose of this arrangement is to establish as much uniformity as possible in the absorption of heat from the air enveloping the absorber. Incidentally this would result in corresponding uniformity in the density of the air affected by the absorber.

Regulating and Measuring the Water Flow

Water for the heat absorber is drawn from a small tank several feet above the ceiling of the chamber, which is filled by water flowing from the city main. An overflow pipe in the tank keeps the water supply at a constant level; and since the level at which the water leaves the absorber is also fixed, the pressure in the system is constant. Under favorable conditions the rate of flow through the absorber is quite regular. At times, however, in cold weather, when a considerable amount of air is dissolved in the water, some of the air that is liberated when the temperature of the water is raised gradually accumulates in the absorber and reduces the rate of flow in an irregular manner. Under these conditions the faster the rate, the more constant it is. For this reason a specially devised rate valve is of only limited service in regulation of the rate of flow, though it has some advantages over the common stopcock.

The water leaving the heat absorber flows into a copper cylinder holding about 3 liters and through a stopcock in the bottom of this into a tank holding about 100 liters. This tank will catch all the water that would leave the heat absorber during a period of at least three hours, in experiments in which the dissipation of heat in the chamber is about 100 Calories per hour, a rate of flow of 350 to 450 c. c. of water per minute, with the temperature of the ingoing water about 16°, having been found quite satisfactory in such circumstances. The large tank rests upon a sensitive platform balance (Pl. XXX) by which the weight of the water is determined to 0.01 kgm. The small cylinder catches the water that flows while the tank is being weighed and emptied.

Regulating the Temperature of Water Entering the Heat Absorber

The temperature of the water entering the heat absorber is so completely under control that it may be kept indefinitely at any desired point within narrow limits, or may be changed rapidly, if necessary, from one point to another. To accomplish this, the water is first cooled to a temperature below that at which it will be used and then brought to the required temperature by electric heating. In these circumstances, when any change in temperature is desired, it is necessary to vary only the heating. The chilled water passes into a device called the preheater, which does the greater part of the heating necessary to warm the water to the desired temperature. The heating effect of this device is adjusted by hand. From the preheater the water flows into the bottom of a large

bottle filled with pieces of pumice as large as will pass through the narrow neck. In this reservoir the water is mixed so that any change in the temperature of that entering the bottle, due, for instance, to fluctuations in the voltage of the current in the preheater, will be dissipated through the mass to such extent that there will be no rapid fluctuations in the temperature of the water leaving the bottle. From this reservoir the water enters the final heater which completes the heating necessary to bring the water to the desired temperature. This device functions automatically and varies the amount of heating it does to accord with the fluctuations in temperature of the water coming from the mixing bottle. From the final heater the water flows into a smaller mixing bottle, from which it passes to the heat absorber.

WATER COOLER

To cool it, the water from the pressure tank is passed through a coil of pipe submerged in cold water, in a tank nearly 1 meter in length by 30 cm. in width and depth and containing 80 to 90 liters of water. The coil consists of nearly 6 meters of iron pipe, of 15 mm. bore, in six parallel rows running from end to end near the bottom of the tank. The water in the tank is chilled by cold brine flowing through a second coil, immersed in the water above the former coil. A small ethyl-chlorid refrigerating machine keeps the temperature of the circulating brine quite uniform. In this manner the temperature of the water leaving the cooling coil is readily kept below that at which it may be needed at any time during an experiment, and fairly uniform, but it can not be regulated by cooling alone as closely as needed for use in the heat absorber.

WATER HEATER ADJUSTED BY HAND

The preheater consists of several coils of electric-resistance wire of different sizes wound upon a thin-walled brass tube about 16 mm. in diameter, from which they are insulated with mica. Outside of this is a similar tube about 26 mm. in diameter, and the annular space between the two and surrounding the resistance coils is filled with sand, so that the heat generated by the electric current in the resistance wire is transmitted rapidly to both tubes. This heater is mounted inside a brass tube 37 mm. in diameter, in such manner that the chilled water, entering the large brass tube, flows in one direction along the outside of the heater and returns along the inside, absorbing all the heat generated in it. By means of plug switches on the base supporting the heater various combinations of the coils may be put into service, as desired, to vary the heating. By the use of this device the temperature of water flowing at a rate of about 1 liter a minute may be increased nearly 10 degrees, if desired, in increments of about 0.25 of a degree.

There are seven resistance coils in the heater, of which four have a resistance of about 340 ohms each. There would be a little less than 0.65 ampere of current flowing in such a coil at 220 volts, which would give approximately 140 watts. To raise 1 degree the temperature of water flowing at the rate of 1 liter per minute requires approximately 70 watts; hence, each of these four coils would increase the temperature about 2 degrees. The resistances of the three other coils are, respectively, about 680, 1,360, and 2,720 ohms, and their output, respectively, about 70, 35, and 18 watts, with corresponding heating effects sufficient to raise the temperature of the water about 1, 0.5, and 0.25 degree.

If these coils were all wound in one tube, the heater would be inconveniently long. Two similar tubes, each 30 mm. long, are used, with the five coils of smaller resistance in one and the two coils of larger resistance in the other. The cold water flows first through the former and then through the latter. The two tubes mounted side by side on the same base may be seen in Plate XXXVI, figure 2, on a board attached to the side of the calorimeter.

WATER HEATER OPERATED AUTOMATICALLY

The final regulation of the temperature of the water for the heat absorber is done in a short tube inclosing a water channel, called the "final heater," which is shown in Plate XXXVI, figure 2, beside the preheater, on the board attached to the side of the calorimeter. In the upper end of the channel is an electric resistance thermometer coil that is connected with an indicator on which may be set the temperature at which it is desired to keep the water entering the heat absorber. In the lower end of the channel is an electric heating coil, in series with which is a rheostat for varying the current in the coil. The slider of the rheostat is adjusted by a screw shaft that is driven by a small electric motor. The water passing through the channel flows directly from the heater to the thermometer. If the temperature of the water flowing over the thermometer differs as much as 0.05 degree from that set on the indicator, the armature of the small motor turns in one direction or the other, depending on whether the water is too cold or too warm, and adjusts the rheostat until the current in the coil is just enough to heat the water to the desired temperature.

The water tube in this device, which is 28 cm. long, has a narrow channel, the cross section being 12 mm. in length and 4 mm. in width and having round ends. It was made by flattening thin-walled copper tubing of an external diameter of 1 cm. At each end the tubing is left circular in cross section and is soldered into a short nipple, which is screwed into one end of a special brass fitting with side outlets. Thin-walled brass tubing 2.5 cm. in external diameter, extending from one nipple to the other, forms a case around the channel, protecting it from

mechanical strain and surrounding it by a small dead-air space which serves to some extent as a heat insulator, protecting it from changes in temperature of the laboratory air. The side openings in the fittings provide an inlet and an outlet for the water.

The electric heater, which is in the lower end of the channel, consists of platinum wire, of 55 ohms' resistance, in a flat coil about 10 cm. long and 9 mm. wide, inclosed in a flat case of thin metal which, with the coil inside, is 10 mm. wide and 2 mm. thick. At one end this flat part of the case tapers into a tube about 3.5 cm. in length and 6 mm. in diameter, in which are the wires carrying electric current to the coil. This heater is inserted in the water channel, through the open end of the fitting, to the depth at which the whole of the resistance wire will be immersed in the water current, and a packing device in the end of the fitting is tightened around the neck of the case to hold the heater in place. In the channel the heater is surrounded by a space 1 mm. across, through which the water flows. Heat generated in the coil is imparted instantly to the water which surrounds the heater in such a thin layer that the temperature of the whole mass of water is very quickly affected.

The electric current flowing in the heating coil is determined by ballast resistance in series with the coil, of which 125 ohms are fixed and 550 ohms variable. For the former an ordinary resistance unit is satisfactory, and for the latter a rheostat of oxidized constantan wire of graduated cross section wound on an insulated light-steel tube has given excellent service. The sliding contact on the rheostat may be moved by hand or by means of a screw shaft (Pl. XXXV, fig. 2). When the total resistance of both the rheostat and the resistance unit is in series with the coil, a current of approximately 0.3 ampere will flow in the coil, the heating effect of which is sufficient to increase by a little less than 0.1 degree the temperature of water flowing through the heater at the rate of 1 liter per minute. When the whole of the rheostat is out of the circuit and only the resistance unit is in series with the coil, the current will be approximately 1.2 amperes, with a heating effect sufficient to increase by a little over 1 degree the temperature of 1 liter of water per minute.

Between these limits the heating effect may be varied in large or small steps, according to the distance the sliding contact is moved along the turns of wire in the rheostat. If the position of the slider is adjusted by hand, any portion of the rheostat, from the total resistance to that of a single turn of the wire, may be instantly put into or out of the circuit. That the temperature of the water may be automatically regulated, however, the position of the slider is adjusted by a screw shaft. A small pulley on the end of the shaft is belted to another pulley on the armature shaft of a small electric motor, that may be caused to run in one direction or the other and for a longer or shorter period, depending upon whether the amount of resistance in the circuit must be increased or decreased and how much. The field coils of the motor are differentially

wound, and the direction in which the armature of the motor will rotate depends upon the windings by which the fields are excited. With current flowing in both pairs of field coils alike the armature will not turn in either direction; but if one pair of coils is shunted, the effect of the other pair predominates, and the armature will rotate. The direction of rotation depends upon the closing of one or the other of two contacts in the circuit of the field windings, thereby shunting one or the other pair of field coils, and the duration of rotation depends upon the length of time the contact is closed.

The contacts are closed by keys which are depressed by cams on a light cam shaft driven by a small motor. The cams rotate continuously, and when a circuit is to be closed an idler swings between one of the cams and the key to be depressed. There are three cams for each key, differing in respect to the time each one presses on the idler, and the duration of contact depends upon which of these cams is engaged. Each idler is mounted on a lever which carries it into position between the cam and the key, the lever being actuated by a pin on the rotating shaft. A galvanometer needle decides which lever is to swing and to which of the three cams it is to be carried. The galvanometer, though incorporated in the device for making contacts, is connected with the indicator on which is set the temperature at which the water is to be kept. The direction and amplitude of deflection of the needle depend upon whether and to what extent the temperature of the water is above or below that set on the indicator. The galvanometer thus governs the direction and extent of motion of the slider on the rheostat which regulates the current in the heating coil in the water channel. The period of the galvanometer is less than 3 seconds, and the cam shaft rotates once every 4 seconds, so that changes in the temperature of the water, when necessary, are made that often, giving practically continuous regulation. With three different degrees of automatic adjustment in either direction, and with the possibility of shifting the slider by hand, the water flow may be quickly brought to any desired temperature and easily maintained. This device for causing the movement of the contact on the rheostat is shown in Plate XXXV, figure 2, which shows also the rheostat and the motor for adjusting the rheostat.

The indicator on which the desired temperature is set, which may be seen in Plate XXXVI, figure 2, at the right of the water heaters, is a special Wheatstone bridge, in one arm of which is the resistance thermometer in the water channel. The resistance of the slide wire of this bridge, which is nearly 45 cm. long, is sufficient to compensate for an upsetting of the balance of the bridge due to the change in resistance of the thermometer that would result from a change of 10 degrees in the temperature of the water, and by means of a coil in series with the wire the amount of balance resistance may be doubled. When the coil and slide wire are in series, the range of the dial is from 12 to 24 degrees;

when the coil is not in series, the reading is from 2 to 12 degrees. In each degree there are 10 subdivisions which are about 4 mm. apart, so that it is possible to change the setting of the indicator by as little as 0.05 degree, and the galvanometer connected with the indicator is sensitive to a change of this magnitude in the balance of the bridge. The resistance thermometer which is in one arm of the bridge is a coil of platinum wire, of approximately 25.5 ohms' resistance at 20° C., the resistance of which changes 0.1 ohm with a change of 1 degree in its temperature. The wire is wound in a flat, narrow coil and inclosed in a very thin silver case, resembling that of the heating coil, and similarly mounted in the upper end of the water channel. The resistance wire very rapidly acquires the temperature of the water flowing in the millimeter space surrounding the case, and changes in resistance instantly follow very small changes in the temperature. The thermometer is a short distance from the bridge, as shown in Plate XXXVI, figure 2, and connected with it by leads that are compensated so that the effect of the resistance of the leads and of change in their resistance due to change in temperature is eliminated.

From the final heater the water flows into a bottle of about 1-liter capacity, nearly full of broken pumice, and then into the heat absorber.

Measuring the Temperature Increase in Heat Absorber

The water that has passed through the heat absorber will have increased in temperature according to its rate of flow and the rate of production of heat in the chamber. The accuracy with which the increase in temperature is determined is of fundamental importance in the measurement of heat generated.

Measurement by Mercury Thermometers

The difference between the temperature of the ingoing and that of the outgoing water was formerly determined by reading two mercury thermometers installed in the water circuit, with the bulb of one in the water just entering the chamber and the bulb of the other in the water just leaving it. The thermometers were as sensitive as it was practicable to employ and were very accurately calibrated. Each had a range of about 12 degrees, with graduations of 0.02 degree, the one in the ingoing water reading from 0° to 12° and the one in the outgoing from 8° to 20°, and by judging the position of the mercury between the graduations the temperature was estimated to 0.01 degree. The observer read the thermometers and recorded the temperatures every two or four minutes, which, in addition to the other duties at the observer's table, was rather tedious and trying. Both thermometers were supposed to be read simultaneously, but as this was impracticable for one observer the two thermometers were read as quickly as possible, and then the

observations were recorded. This method afforded opportunity for errors in fundamental data, some of which might be obvious, but most of which would not be detected.

MEASUREMENT BY ELECTRIC-RESISTANCE THERMOMETERS

To relieve the observer of the tedium of these observations, and especially to eliminate as much as possible of the personal element from the measurement, the mercury thermometers were replaced by a device for measuring the increase in the temperature of the water by the difference in electrical resistance of two coils of wire in the water circuit. Atwater and Rosa (4, p. 25; 5, p. 151) employed a device of this character in their original calorimeter, but did not develop it to measure temperature differences with the same degree of accuracy as the one here described. The latter device comprised two special resistance coils, a special Kohlrausch bridge, a sensitive galvanometer, and a lamp and scale for reading the deflections of the galvanometer. The specially mounted resistance coils, called the "bulbs," were inserted in the water line where the bulbs of the mercury thermometers had been and were connected with the special Kohlrausch bridge on the observer's table, the two coils being in opposite branches of the bridge circuit, with the slide wire between them. The reflecting D'Arsonval galvanometer by which the bridge was shown to be balanced was suspended in such a position that the scale on which the deflections of the galvanometer were read was on a level with and directly in front of the eyes of the observer sitting at the table. The movement of the galvanometer was indicated by the movement of a vertical line of light along the scale, the light from a straight-filament electric lamp being reflected by the mirror of the galvanometer. To determine the difference in the temperature of the two coils, it was merely necessary to move the battery circuit contact along the slide wire of the bridge until the line of light was at the center of the scale, showing that the bridge was balanced. The reading of the bridge scale was then recorded. To balance the bridge and read its scale was much more convenient than to read the mercury thermometers, and only one record was involved.

Several types of resistance-thermometer bulb were tried in connection with this device. In one, insulated resistance wire was incased in a coil of small-bore lead tube, which was immersed in an enlargement in the water channel. This proved unsatisfactory for several reasons. One was that it did not respond quickly enough to changes in the temperature of the water, owing probably to poor thermal contact between the wire and the tube; and the mass of metal in the tube also tended to increase the lag. Another was that the space in the lead tube was not deprived of water vapor, and this eventually moistened the insulation of the resistance wire, so that a short circuit was established between the wire and the

tube sufficient to ruin the bulb for accurate measurement of temperature. After the bulbs had been in use a short time they would produce an electromotive force as if they were primary or secondary cells.

In another type of thermometer bulb the resistance wire was inclosed in a thin-walled small-bore copper tube, which was filled with Wood's metal to exclude moisture from the tube and to render the thermometer more sensitive by increasing the conduction of heat to the wire. This bulb did not prove satisfactory because, though the thermal conductance from the water to the wire may have been improved, the sensitiveness of the thermometer was not, the mass of metal apparently causing a lag in response to temperature change. Furthermore, the Wood's metal apparently did not completely exclude moisture, for ultimately the wire in this thermometer also became short-circuited with the metal. Another serious objection was the possibility that the resistance wire might be stretched by the unequal expansion of the metal in which it was embedded.

The bulb which was finally used with utmost satisfaction was constructed in accordance with the specifications of the one developed by Dickinson and Mueller (11, 12, 13) in connection with investigations on calorimetry at the United States Bureau of Standards, which was designed especially for use in determining the temperature at a definite point of liquid flowing in a tube in a continuous-flow calorimeter. The bulbs were designed especially to combine constancy, freedom from lag, and intimate contact with the entire water flow. The platinum resistance wire was wound on a thin strip of mica, and this coil, laid between two similar mica strips, was inclosed in a flat sheath of thin silver which pressed the mica insulating strips firmly against the resistance wire, thus affording opportunity for rapid conduction of heat between the case and the wire. The silver case terminated at the top in a tube which was sealed to a glass tube, on the end of which was a bulb containing phosphorus pentoxid, the purpose of which was to exclude moisture from the space in which the resistance wire was inclosed. The flat part of this bulb, which was about 10 cm. long, 10 mm. wide, and 1 mm. thick, and contained the sensitive part of the thermometer, was inserted in a brass tube with a constricted channel, like that for the final heater described on page 319, so that the sensitive portion of the thermometer was surrounded by a space about 1 mm. across; and water flowing through this space was thus brought into intimate contact with the thermometer, which very rapidly acquired the temperature of the water and responded instantly to changes in temperature and integrated stream lines of temperature, if any existed. The two thermometers, one in the ingoing and the other in the outgoing water, had exactly the same resistance, about 25.5 ohms at 20° C., and the same coefficient of change of resistance with change in temperature, about 0.0039 per degree for the range of temperature in which they would be used, the resistance change of each thermometer being 0.1 ohm per

degree. With the regular leads to each thermometer from one branch of the bridge circuit was a compensating loop from the opposite branch of the bridge, to balance the resistance of the leads in both branches of the circuit, and to eliminate the effects of changes in the resistance of the leads due to changes in their temperature and of thermal electromotive forces. All connections in the bridge circuit were soldered—that is, there were no contact connections; hence, no possibility of error due to contact resistance in any part of the circuit.

The special Kohlrausch bridge was designed to measure any difference as large as 10 degrees or as small as 0.01 degree in the temperature of the water as it entered and as it left the heat absorber. The slide wire of the bridge, which was about 4.5 meters long, consisted of 10 turns of manganin wire wound spirally on a cylinder of marble about 15 cm. in diameter. The battery-circuit contact, which balanced the bridge by the adjustment of its position on the slide wire, was mounted on the inside of a hood surrounding the cylinder, which, when rotated, moved up or down on a threaded center post. Since the contact was in the battery circuit, whatever contact resistance there might be had no effect on the balancing point of the bridge. The resistance of the total calibrated portion of the slide wire was sufficient to balance the bridge when the resistance of the two thermometer coils differed by as much as 1 ohm, which would occur with a difference of 10 degrees between the temperature of the ingoing and that of the outgoing water. With one rotation of the hood the contact was moved over sufficient of the slide wire to balance a difference of 0.1 ohm or 1 degree in the thermometers. On the edge of the hood was a scale with 200 divisions, each corresponding to a little over 2 mm. of the slide wire. A movement of the contact on the wire the space of two divisions would be sufficient to balance a difference of 0.001 ohm or 0.01 degree in the thermometers.

The sensitivity of the galvanometer was sufficient to indicate a change of even one division in the bridge setting, equivalent to 0.005 degree in the temperature of the thermometer. With the usual current of 0.03 ampere in each half of the bridge, a change of 0.001 ohm would be indicated by a deflection of several millimeters on the galvanometer scale. A current of 0.03 ampere flowing in each resistance thermometer would not cause an increase of 0.005 degree in the temperature of either, when immersed in water flowing at the rate of 200 c. c. per minute, which would be not over half the common rate in the experiments.

Provision was made for checking the results obtained with the electric-resistance thermometers. The second type of resistance bulb mentioned above was constructed so that the bulb of the mercury thermometer formerly used could be inserted into the bulb of the resistance thermometer, and the temperature differences determined by both sorts of thermometers at the same time. The results obtained by the two

methods before the resistance coils became short-circuited were always in very satisfactory agreement, but this was hardly a sufficient test of the accuracy of the resistance method, because the measurement of temperature difference by the electric-resistance thermometers is much superior to that by the mercury thermometers in sensitivity and precision. With the third type of resistance bulb a more satisfactory method of checking was provided. A differential thermoelement, with several junctions of copper and constantan wire in each end inclosed in thin glass tubing, was mounted with one end in the water just leaving the ingoing thermometer and the other end in the water just entering the outgoing thermometer. The terminals of the thermoelement were connected with binding posts on the observer's table, from which connection could be made with a potentiometer, by means of which temperature differences could easily be measured to an accuracy of 0.01 degree. Measurement of the increase in temperature of the water flowing in the heat absorber by means of this apparatus afforded a real check on the measurement with the resistance thermometer.

MEASUREMENT BY TEMPERATURE DIFFERENCE RECORDER

As a matter of fact, this method of measurement could be employed instead of the resistance-thermometer method when the readings are to be made and recorded by the observer. Either method was more convenient and decidedly more sensitive than the mercury thermometers, and by use of it the temperature difference was actually measured to 0.01 degree, whereas in reading the mercury thermometers the temperature was only estimated to 0.01 degree. The particular advantage in the resistance thermometers was in the opportunity to use with them a device which gives automatically a practically continuous record of the difference between the temperature of the water entering and that of the water leaving the heat absorber. A device of this character which has been employed for five years in the investigations with the present respiration calorimeter has proved very satisfactory indeed and relieves the observer of a considerable amount of drudgery, while it entirely eliminates the possibility of error due to personal inaccuracy in recording data regarding the temperature differences.

Like the resistance thermometers described above, the two coils used in this device have the same resistance, approximately 25.5 ohms, at the same temperature, and the same change in resistance with the same change of temperature, but the bulbs differ somewhat in mechanical construction from the earlier type. The platinum resistance wire is not in a thin, flat coil in a flat sheath, but is in a helical coil in a narrow annular space between two metal tubes with thin walls. The wire is wound upon the inner tube, and the outer tube fits close against it, an electrical insulation of thin sheet mica separating the wire from each tube. The space between

the tubes is tightly closed at each end, the leads from the resistance wire being carried out through a small tube attached to the tube surrounding the wire. As in the flat-type thermometer, this small tube terminates in a bulb containing phosphorus pentoxid, to keep the annular space free from moisture. The cylindrical shell inclosing the resistance wire is mounted in a brass tube which provides a water channel so designed that the water flowing in it passes inside the inner and outside the outer of the tubes incasing the wire, which is thus brought into intimate contact with all the water flowing through the thermometer, and responds instantly and accurately to changes in its temperature. Because of the design of the thermometer and the manner in which it is mounted in the walls of the chamber, the usual fluctuations of the temperature of the air adjacent to the case of the thermometer introduce no error in the measurement of the temperature of the water flowing in the bulb. One of these thermometers is placed in the incoming water pipe so that it will be at the temperature of the water just as it passes through the copper wall, and the other is similarly placed in the outgoing water pipe.

The two thermometer coils are in the corresponding arms in opposite branches of the circuit of a special Wheatstone bridge (Pl. XXXV, fig. 2), which may be accurately balanced for inequalities in resistance of the coils as small as 0.001 ohm and as large as 1 ohm, resulting from a difference of 0.01 degree and of 10 degrees, respectively, between the temperature of the water entering and that of the water leaving the heat absorber. The total resistance of the slide wire of the bridge will compensate for an inequality of 0.2 ohm in the resistance of the coils which results from a difference of 2 degrees in their temperature. If there is no difference in the temperature of the water in the two thermometers, the bridge is balanced with the battery circuit contact at the low end of the wire, while if the temperature of the water leaving the heat absorber is 2 degrees higher than that of the water entering it, the bridge is balanced when the contact is at the upper end of the wire. To compensate for inequalities due to temperature differences greater than 2 degrees, eight coils of manganin wire in series are arranged so that any number of them may be connected in series with the slide wire, thus shifting the position of the contact on the wire at which the bridge may be balanced and altering the significance of the balance point in temperature difference. The lower end of the wire may thus be made to correspond to any whole number of degrees of temperature difference between 0 and 8, with the upper end 2 degrees higher in each case. The coil and slide wire are joined by means of a heavy copper link, with one end in the mercury cup in which one end of the slide wire terminates and the other end in a similar cup in which an end of the extension coil terminates.

The slide wire of the bridge is incorporated in a mechanism (Pl. XXXV, fig. 2) which automatically balances the bridge for inequalities of resist-

ance in the thermometer coils and records the balancing operations in terms of temperature difference and time. The wire is mounted in a bar which supports and guides a slider carrying the battery circuit contact point along the slide wire. The slider is actuated by a small electric motor, the direction and extent of motion of the slider being governed by the direction and the amplitude of deflection of the pointer of a galvanometer which is connected between the two branches of the bridge circuit, and is incorporated with the slide wire in the mechanism which balances the bridge. The direction in which the pointer will swing depends upon whether the inequality of resistance of the thermometer coils increases or decreases—that is, whether the difference between the temperatures of the water in the thermometers grows larger or smaller. For example, if the temperature of the outgoing water rises or that of the ingoing water falls, the pointer will swing so as to cause the slider to move toward the high end of the wire. The amplitude of deflection of the pointer depends upon the magnitude of the inequality of resistance of the thermometer coils. The bridge and galvanometer are sensitive to very small temperature changes in the thermometer. With the measuring current of 0.05 ampere in each thermometer coil a difference of 0.0005 ohm in the resistance of the two coils, which results from a difference of 0.005 degree in the temperature of the water in the thermometer, causes a deflection of the pointer sufficient to influence the position of the contact on the slide wire. With a measuring current of 0.05 ampere each coil would dissipate about 0.06 watt, which would be sufficient to raise the temperature of the thermometer 0.005 degree if the water were flowing through it at a rate of only 200 c. c. per minute; but since the rate of flow is generally twice as great, the effect of the measuring current on the temperature of the bulb is negligible.

Each time it changes the position of the battery circuit contact point on the slide wire the automatic shifting mechanism moves the slider one of three different distances in either direction, according to the amplitude of deflection of the galvanometer pointer. With the smallest change of position the contact is moved along the wire sufficiently to balance the bridge for inequality of resistance in the thermometers due to differences of less than 0.01 degree in the temperature of the water. The medium change balances differences of resistance equivalent to differences of nearly 0.03 degree in temperature, and the large change corresponds to temperature differences of 0.05 degree. The shifting mechanism functions every 7 seconds; hence, it will keep the bridge in balance for any change in temperature difference not exceeding 0.4 degree per minute; but inasmuch as the position of the contact point on the slide wire may be easily adjusted by hand for any inequality of resistance within the range of the instrument, any alteration in temperature difference may be followed.

As the slider moves back and forth on the bar which supports the slide wire, it carries a pen which draws a curve on ruled paper by which the movement of the contact point on the slide wire is expressed in temperature. The total width of the paper scale, 25 cm., represents a difference of 2 degrees between the temperature of the water entering and that of the water leaving the heat absorbers, and corresponds exactly to the length of the slide wire by which the bridge is balanced for the inequality of resistance in the thermometer coils resulting from such a temperature difference. The temperature difference indicated by the position of the pen on the paper scale coincides with that to which the position of the contact point on the slide wire is equivalent. The paper scale is ruled with 100 lines, each representing 0.02 degree, and as the distance between the lines is 2.5 mm., the curve may easily be interpreted to 0.01 degree. The paper is moved forward at a very regular rate, approximately 7.5 cm. per hour, by the motor which moves the slider, the speed of the motor being regulated by a governor so that it is uniform, even with wide fluctuations in voltage of the current by which the motor is driven. Since the necessary changes in the position of the slider are made every 7 seconds, the curve gives a practically continuous record of the temperature difference.

The difference between the temperature of the water as it enters and that as it leaves the heat absorber may thus be easily read at any instant to 0.01 degree. The accuracy of the measurement of temperature difference by the apparatus may be tested at any time, even during the course of an experiment, without interfering with the record, and such tests are made at frequent intervals. In the water channel in the center of each resistance-thermometer bulb is the end of a differential thermoelement of 0.125 mm. copper and constantan wires, having 11 junctions in each end, inclosed in 4-mm. glass tubing, with thin wall. The element remains permanently in position, though it may be easily removed if necessary. The terminals of the element are joined by insulated 1-mm. copper wire to binding posts on the observer's table, from which connection can be made with a potentiometer whenever a test is to be made. With this differential thermoelement, which has been calibrated over a wide range of temperature at the United States Bureau of Standards, an electromotive force of over 4.5 microvolts results from a difference of 0.01 degree in the temperatures of the two ends. By means of the potentiometer and galvanometer with which it is employed, an electromotive force of half that magnitude is easily measured; consequently temperature differences may be measured by it to an accuracy at least as good as 0.01 degree. Measurements made with this apparatus therefore serve to indicate the accuracy of those with the recorder. The agreement of results obtained by the two methods of measuring the increase in the temperature of the water flowing through the heat ab-

sorber is shown in Table I, which summarizes data obtained in an alcohol check test (see p. 342) of the calorimeter made in January, 1915, which continued for two consecutive periods of three hours each.

TABLE I.—*Comparison of data for heat measurement obtained by use of temperature difference recorder and of thermoelement with potentiometer*

Time.	Water flow.	Temperature difference.		Heat computed from measurement.	
		By recorder.	By potentiometer.	By recorder.	By potentiometer.
	Kgm.	*Degrees.*	*Degrees.*	*Calories.*	*Calories.*
1 hour	20.53	3.99	4.01	81.9	82.3
Do	21.20	3.97	3.96	84.2	84.0
Do	23.00	3.73	3.73	86.0	86.0
Total	.....	.....	.....	252.1	252.3
1 hour	23.00	3.68	3.68	84.6	84.6
Do	23.21	3.57	3.54	82.9	82.2
Do	22.72	3.67	3.67	83.4	83.4
Total	.....	.....	.....	250.9	250.2

In order that the recording device may continue to measure temperature differences with the accuracy required, not only must the bridge be sensitive to a change as small as 0.002 per cent in the resistance of the thermometer coils, but also the resistances of the various parts of the bridge circuit other than the thermometers must not change as much as 0.003 per cent. Provision is made for testing the component parts of the bridge by the substitution of duplicate parts, which are mounted with the ratio coils of the bridge in a check box, and tests of this character are made at frequent intervals. After the apparatus had been in use for a short time a very slight change in one of the ratio coils was detected and corrected. Since that time the bridge has remained remarkably constant. It is possible also to test with the check box and recorder whether the thermometer coils remain alike in resistance at the same temperature. Provision is made in the check box for correcting slight inequalities in them by a variable shunt across a coil of small resistance in series with one of the thermometers.

PREVENTING TRANSFERENCE OF HEAT THROUGH THE WALLS OF THE CHAMBER

In order that the quantity of heat produced in the chamber may be accurately measured, either there must be no increase or decrease in it due to the passage of heat through the walls of the chamber, or if heat is thus added or subtracted, the quantity must be determined and allowance made for it. This calorimeter is constructed and operated in accordance with the method employed in the original calorimeter of

Atwater and Rosa, to prevent gain or loss of heat through the walls, though with modifications in details which make the present apparatus exceedingly sensitive, while easy to operate. The copper wall[1] of the chamber is duplicated by a wall of zinc attached to the outside of the iron framework which supports the copper wall, as explained on page 303, and the temperature of the zinc wall is regulated to accord with that of the copper wall in such manner that the thermal conditions of the two walls will be in equilibrium with each other. When the temperature of the zinc wall is the same as that of the copper wall, the quantity of heat transmitted from each wall to the other is the same, so that neither wall actually gains heat from the other. The effect of such a condition on the quantity of heat in the chamber would be the same as if no heat were to pass from either wall to the other in either direction. If the temperature of the zinc wall is above that of the copper wall, the quantity of heat passing from the zinc to the copper is greater than that in the reverse direction—i. e., the copper wall will gain heat from the zinc wall, some of which, at least, it will transmit to the air of the chamber. Conversely, if the temperature of the zinc wall is below that of the copper wall, the former will gain heat from the latter, some or all of which the copper wall has derived from the air of the chamber. If the quantity of heat which the copper wall has gained from the zinc wall is counterbalanced by an equal quantity gained by the zinc wall from the copper wall, the effect on the measurement of the quantity of heat produced in the chamber is the same as if no heat had been transferred from either wall to the other. This counterbalancing may be accomplished by keeping the temperature of the zinc wall above or below that of the copper wall, as need be, to the same degree and for the same length of time that the conditions were reversed. For this purpose means are provided for determining when the zinc wall is warmer or colder than the copper wall, and for heating and cooling the zinc wall as is found necessary.

Detecting Differences in Temperature of the Double Metal Walls

Thermoelectric thermometers are used to detect any difference between the temperature of the zinc wall and that of the copper wall. Differential thermoelements are installed between the two walls, with the junctions at one end of each element close to the outer surface of the copper wall, while those of the other end are in the plane of the zinc wall, and the terminals of the elements are connected with a sensitive galvanometer. The direction of the deflection of the galvanometer indicates whether the zinc wall is warmer or cooler than the copper wall—i. e., whether to warm or to cool the zinc wall.

Each thermoelement consists of four copper-constantan couples made of bare hard-drawn wire about 1 mm. in diameter (No. 18, American gauge). In making the junctions, the copper and constantan wires were put end

[1] As used in this section, the term "wall" may include the ceiling and the floor as well as the side walls.

to end and joined with silver solder. The wires were then bent at the junctions into a grid, with the parallel lines about 5 mm. apart and with copper and constantan alternating. Each constantan wire and three of the five copper wires are about 7 cm. long, so that the distance between the two opposing sets of junctions is the same as that between the copper wall and the zinc wall. The two other copper wires, which are at opposite ends of the series, are longer, to form leads for the element, as explained below.

Wire of the size stated was used chiefly because it was most readily available and seemed quite well adapted to the type of element constructed. Theoretically, a small wire would be preferable, because of smaller thermal conductance, but the support in which each element is mounted probably greatly delays change in temperature of the wires between the junctions, while affording opportunity for rapid change at the junctions. This support consists of a hard maple rod or spindle about 10 cm. in length and 15 mm. in diameter. A recess 8 mm. wide and 2 mm. deep is cut around the spindle 3 cm. from one end, and in the surface are 10 equally spaced longitudinal slots, each nearly 1 mm. wide and 2 mm. deep. The five copper and four constantan wires which, joined alternately in series in a grid, as described above, comprise the four differential thermocouples of an element, were forced into these slots until they were about a millimeter below the surface of the wood and to that extent were protected against contact with the metal sleeve and thimble by which the thermoelement is supported in the walls, as explained below. By means of a cut between two adjoining slots near the center of the spindle the copper wire at one end of the series is doubled back and extends parallel with the copper wire at the other end of the series, the two projecting from one end of the spindle and providing terminals for the element. The spindles with the wires thus embedded were boiled in paraffin for two or three hours, so that they would not swell or shrink with changes in the humidity of the air.

The temperature of the wires thus embedded in the spindle is probably that of the spindle and therefore changes slowly—i. e., the temperature gradient in each wire is quite like that of the others in the element and is relatively constant for considerable periods. On the other hand, the junctions between the copper and the constantan wires are not embedded, one series of four alternate junctions projecting into the air at one end of the spindle, while the series of opposing junctions projects into the air in the recess near the other end of the spindle, so that changes in the temperature of the air surrounding them affect the junctions quickly.

To keep each element in place between the two metal walls a short copper sleeve is passed through a hole in the zinc wall, the sleeve being soldered to the zinc at the edge of the hole to insure good thermal conductance; and directly opposite, with its open end facing that of the sleeve, a short copper thimble is firmly soldered to the outer surface of

the copper wall. A spindle is pushed through the sleeve and into the thimble until the junctions projecting from its inner end are very close to the bottom of the thimble, actual contact being prevented by the adjustment of a small screw in the end of the spindle. A change in the temperature of the copper wall immediately affects the temperature of the thimble attached to it, and consequently that of the junctions within the thimble. The junctions in the recess at the other end of the spindle are within the sleeve attached to the zinc wall, and any change in the temperature of the zinc wall affects the sleeve and, hence, the temperature of the junctions within it. Since both the sleeve and the thimble are short, neither affects the temperature of the wire in the elements any considerable distance from the junctions. The sleeve, however, projects slightly either side of the zinc wall, so that it will surround the junctions, even when they might come inside or outside the plane of the zinc, because of inequalities of distance between the two metal walls.

A short section of the spindle, between the recess and one end, projects from the outer end of the sleeve in the zinc wall and provides a firm stay for the terminals of the elements.

There are 95 such thermoelements distributed in the walls of the chamber. If they were equally spaced there would be one for each 4.5 dm. square of surface; but since the temperature of the chamber would tend to vary more at the top than at the bottom, more elements were installed in the upper than in the lower parts of the chamber to increase the sensitivity and integrate a larger number of sections of the walls. There are accordingly 16 elements in the ceiling and 10 in the floor. In the sides are five rows, with 14 elements in each row except the first one from the top, from which one is missing because the space in which it would be located is occupied by the window. The five rows are not quite equally separated, the two upper rows being slightly nearer together than the three lower ones, in accordance with the idea that the temperature of the upper section would tend to vary more than that of the lower one. These thermoelements are joined in groups in such manner that a difference between the temperature of the copper wall and that of the zinc wall may be detected in certain portions of the walls without regard to conditions in other parts. One group includes the 16 elements in the top; another the 28 elements in the two upper rows of the sides, called the upper zone; a third, the 42 elements in the three lower rows of the sides, called the lower zone; and a fourth group, the 10 elements in the bottom. The thermoelements in each group are connected in series by heavily insulated No. 18 copper wire, and the same sort of wire leads from the terminals of each group to a multiple point switch on the observer's table by which the groups may be connected successively with the galvanometer. It is also possible to connect all 95 thermoelements in series as a whole with the galvanometer and thus observe the average difference between the temperature of the copper wall as a whole and that of the zinc wall as a whole.

In the multiple-point switch the leads from the different groups of thermoelements terminate in a double row of studs arranged in segments of concentric circles, and the galvanometer leads terminate in two metal rings concentric with the studs (Pl. XXXIII, fig. 1). Metal strips, passing through a vertical shaft at the center of the circles, complete the circuit from studs to rings, the ends of the strips being bent to touch edgewise. On turning the shaft by means of the handle at the top, the strips are shifted from one pair of studs to another, thus connecting the different systems with the galvanometer. The switch includes studs not only for the thermoelement groups described above, but also for resistance thermometers described beyond, so that the same galvanometer will serve for several systems.

The galvanometer with which the electromotive forces in the thermoelement circuits are detected is a reflecting instrument of the D'Arsonval type, with a coil resistance of 39 ohms. When critically damped, it has a period of 7 seconds, and a sensitivity such that an electromotive force of approximately 2 microvolts in either circuit will cause a deflection of 1 mm. on a scale 1 meter from the mirror of the galvanometer.

With this galvanometer the number of thermoelements in each circuit is sufficient to cause a fairly large deflection when the temperature of the zinc wall is only slightly different from that of the copper wall. In the bottom section, for example, there are 10 thermoelements, the smallest number in any section, each with four differential couples, and each couple having a thermal electromotive force of close to 40 microvolts per degree of temperature difference between the junction at one end and that at the other. All 40 couples being in series, there would be a total electromotive force of 1,600 microvolts for an average difference of 1 degree between the temperature of the copper wall and that of the zinc wall in this section, or 16 microvolts for an average difference of 0.01 degree. Since an electromotive force of about 2 microvolts will cause a deflection of 1 mm., a difference of 0.01 degree would cause a deflection of at least 7 mm. It is easy to read a deflection of less than 1 mm.; consequently the effect of a temperature difference as small as 0.001 degree between the junctions at opposite ends of the thermoelements in this may be observed. The effect of such a difference in the other sections would be greater, because of the larger number of elements; the 16 in the top would cause a deflection of more than a millimeter; the 27 in the upper zone of the sides about 2 mm.; and the 42 in the lower zone more than 3 mm.

Controlling the Temperature of the Zinc Wall of the Chamber

The temperature of the zinc wall is raised or lowered by heating or cooling the air confined in the narrow space adjacent to the outer surface of the zinc, which has a corresponding effect on the wall. Parallel with the wall, and about 4 cm. outside of it, is a wall of cork board 38 mm. thick,

which is such a good heat insulator that appreciable changes in the temperature of the air in the laboratory affect the temperature of the air confined in the spaces between the cork board and the zinc wall very slowly. The temperature of the air in this space adjacent to the zinc wall is raised by converting electrical energy into heat in a resistance wire that is strung on porcelain insulators attached to the wall; and it is lowered by passing cold water through small-bore brass pipes supported by small iron hooks screwed to the framework to which the wall is attached. Short sections of both pipes and wires and the method of attaching them to the wall are shown in Plate XXXIV, figure 2.

By wooden strips extending from the metal wall to the cork board, the air space surrounding the zinc wall is divided into sections corresponding with the top, the upper and lower zones of the sides, and the bottom of the chamber, as already described in the case of the thermoelements in the walls. A portion of one strip is shown in Plate XXXIV, figure 2. Each section has its own heating device and cooling device, so that the temperature of the corresponding portion of the zinc wall may be controlled independently of the conditions in any other space, and the possibility of heat entering the chamber in one part of the wall and leaving it in another is prevented.

The current of water for cooling the zinc wall flows through brass pipe of about 7 mm. bore (so-called ⅛-inch pipe). In the top and bottom sections the pipe extends forward and back from end to end for the whole width of the space, the successive lengths of pipe being about 15 to 20 cm. apart. In the upper and lower zones the pipe extends continuously around the four sides of the walls, the succeeding turns of the coil being about as far apart as those in the other sections. This furnishes ample cooling effect, which can be regulated by varying the temperature of the water flowing in the pipe, or the rate of flow, or both. The inlet ends of the four pipes are connected with the feed-water pipe, with the small brass needle valves used for regulating the flow in the cooling coils close together and convenient to the operator at the observer's table (Pl. XXXVI, fig. 2). The outlet ends of the coils are also brought together in a funnel below the regulating valves, so that the effect of the valves on the rate of flow may easily be seen.

The electric current for heating the zinc wall is conducted by a non-corrosive wire of a high carrying capacity, the resistance of which is about 3.5 ohms per meter. In each space the wire is distributed, as the cooling coils are, over practically the whole surface of the zinc, the successive lengths of wire extending from one end of the space to the other, about 15 cm. apart. The amount of wire in each space is such that without regulation of the current in it the heating effect would be greater than necessary. With the proper ballast resistance in series with each heater the heating effect in each section may, if desired, be made proportional to the area of zinc to be heated. In the upper zone

of the sides, for example, there is an area of about 5.8 square meters. The total resistance of the wire in the space is 143 ohms. In series with this wire but exterior to the space is a resistance unit that may be varied according to the need for current. If a unit of 200 ohms' resistance were used, there would be a little over 0.64 ampere of current flowing in the heating wire, the pressure of the current being 220 volts; and the total amount of electrical energy (I^2R) dissipated in the 143 ohms of wire would be nearly 59 watts, or roughly 10 watts per square meter of surface of zinc. Similarly, the area of the lower zone is about 8.9 square meters, and the resistance of the wire in it is 195 ohms; with an exterior unit of about 125 ohms in series with the heating wire, the amount of energy dissipated in the latter would be about 92 watts, or slightly over 10 watts per square meter. There are close to 2.9 square meters in the top section and the same area in the bottom, and in each of these sections is a heating coil of 117 ohms; with an exterior unit of 325 ohms in series with it, the current in each heater would approximate 0.5 ampere, and about 29 watts would be dissipated in the 117 ohms of resistance wire, or 10 watts per square meter.

In controlling the temperature of the zinc wall cold water is kept flowing continuously through the brass pipe in the air space outside of it at such a rate of flow, depending upon the temperature of the water, that the temperature of the unheated air would be lower than that at which the wall is to be kept. With a constant flow of water the temperature gradient along the pipe is quite flat in comparison with what it would be if the rate of flow were increased or decreased as the air would need to be cooled or heated; in other words, the cooling effect is fairly uniform throughout the length of the pipe. At the same time electric energy is converted into heat in the resistance wire until the air is warmed enough to bring the wall to the desired temperature. Since this dissipation of heat is equal in all parts of the wire, the total mass of air in the space is quite uniformly heated. Under these conditions to change the temperature of the wall requires only an increase or decrease of the current in the resistance wire, according to whether the wall is to be heated or cooled, which involves merely the adjustment of a rheostat in series with the wire, so that regulation is easily and quickly effected. A rheostat of oxidized constantan wire wound on an enameled metal tube and having a sliding contact passing over successive turns of the wire, with a resistance of about 980 ohms and a current-carrying capacity of 1 ampere, is in series with the resistance wire comprising the heating coil in each section. The four rheostats for the different air sections to be controlled are attached vertically to an asbestos slab at one end of the observer's table, as seen in Plate XXXVI, figure 1, with the sliding contacts in easy reach of the operator reading the galvanometer deflections.

The temperature of the zinc wall is kept as nearly as possible like that of the copper wall, so that the deflections of the galvanometer connected with the differential thermoelements in the walls are as close as possible to 0. Even under the most favorable conditions it is hardly practicable to keep the two walls so uniformly alike that there will be no deflection at any time, because the temperature of the copper wall, however well regulated, does vary to some extent, and it is not possible to anticipate the change. It is possible, however, to keep the deflections most of the time so small that any error introduced by the temperature differences which they indicate would be insignificant. As explained on page 334, the number of thermoelements in each section of the walls and the sensitivity of the galvanometer are such that a very small difference between the temperature of the copper wall and that of the zinc wall would cause a fairly large deflection; hence, a very small deflection really means a practical identity of temperature of the two walls. When the rate of production of heat within the chamber is quite uniform and the rate of abstraction of heat is so nearly like it that the temperature conditions within the chamber are quite constant, the temperature of the zinc wall may be kept so nearly like that of the copper wall that the deflection will not exceed 5 mm. and will generally be less. A deflection of that magnitude would indicate for the bottom section a difference not greater than 0.005 degree between the average temperature of the copper floor of the chamber and that of the zinc wall outside of it; for the other sections it would indicate still smaller differences. The amount of heat gained by either wall from the other with such small differences is of little importance in comparison with the total amounts usually measured in the chamber. In an experiment with a variable heat production, as would be the case with a man moving and quiet by turns, such a close balance could hardly be maintained at all times, though the deviation need not greatly exceed 5 mm. for any considerable periods. Furthermore, it is possible to make the deflections in one direction equal to those in the other direction for equal short periods, so that whatever heat may be gained by the copper wall from the zinc wall during one period is counterbalanced by that gained by the zinc wall from the copper wall during the succeeding period, in which case there is no actual increase or decrease of the quantity of heat in the chamber for the total time of the two periods due to an exchange of heat between the walls.

In order that the walls controlled in the manner described shall be heatproof, their temperature and that of the iron structure between them must be the same. The temperature of the copper wall, and consequently that of the zinc wall, is governed by that of the air in the chamber; but the two walls may be brought into thermal equilibrium at a temperature above or below that of the framework, in which case the quantity of heat in the chamber would probably be affected by the

mass of iron with its large thermal capacity and high conductivity, the magnitude of the effect depending upon the difference between the temperature of the iron and that of the air in the chamber. To avoid error from this source in the measurement of the heat generated in the chamber it is very essential not only to keep the temperature of the walls of the chamber and that of the air of the chamber as nearly alike and as constant as possible during the period in which the measurements are made, but also to be certain that at the beginning of the period the temperature of the iron structure is identical with that at which the walls and air are to be kept. To this end the regular experimental period must be preceded by a period in which the walls and their supporting structure are brought to the desired temperature. The length of this period depends upon the temperature conditions of the walls when it begins, but it is shortest when the temperature of the walls and framework is kept under control by means of a thermoregulator in the chamber during the periods in which experiments are not in progress. With care and attention to the details outlined it is possible to prevent gain or loss of heat through the walls of the chamber, but the amount of attention and manipulation necessary to avoid error because of the metal would be avoided if the framework were constructed of material having small thermal capacity and poor conductivity. Such a change would be made in reconstructing the calorimeter.

PREVENTING GAIN OR LOSS OF HEAT IN THE AIR ENTERING AND LEAVING THE CHAMBER

Provision is also made against loss or gain of heat in the circulating air. A thermoelement of 40 couples is installed with one end of each couple in the incoming air just as it enters the chamber and the other end in the outgoing air just as it leaves the terminals of the element leading to the multiple point switch on the observer's table, by which it may be connected with the galvanometer. Any difference between the temperature of the ingoing air and that of the outgoing air indicated by the galvanometer is corrected by heating or cooling the ingoing air as needed. A copper tube of small bore is coiled tightly on the brass pipe that conducts the air into the chamber for a distance of about 30 cm. just before the pipe enters the wall, and through this coil water runs continuously, tending to keep the air too cool. Adjacent to this, also on the brass pipe, is an electric heating coil of about 800 ohms' resistance, which warms the air to the desired temperature. To change the temperature of the air, only the current in the heating coil is varied. In series with this coil is a tube rheostat of about 2,500 ohms' resistance by which the current in the resistance coil and, hence, its heating effect are regulated, the position of the sliding contact being adjusted until the galvanometer indicates that the temperature of the ingoing air is the

same as that of the air leaving the chamber. This rheostat is mounted on the end of the observer's table beside those for controlling the temperature of the zinc wall.

ALLOWANCE FOR CONDITIONS AFFECTING THE HEAT OF THE CHAMBER

Any passage of heat into or out of the chamber through the walls or in the ventilating air current being prevented, the sum of the quantity of latent heat in the water vapor of the outgoing air and that of sensible heat removed by the water circulating in the heat absorber would equal that actually produced in the chamber if there were no change in the temperature of the walls or in that of any objects confined within them. Under ordinary conditions, however carefully the rate of abstraction of heat from the chamber has been regulated to accord with that of production, temperature changes can not be absolutely avoided, so they must be measured and allowance made for them.

Change in Temperature of the Metal Walls

If the temperature of the copper wall is lower at the end of a given period than it was at the beginning, and the temperature of the zinc wall has been kept identical with that of the copper wall throughout the period, a certain amount of heat has been imparted to the air of the chamber by the copper wall during the period; or, conversely, if the copper wall is warmer at the end of the period, some heat has been absorbed from the air by the wall. To ascertain how much allowance must be made for the heat involved in such changes, it is necessary to determine the temperature of the copper wall at the beginning and the end of the period and to know how much heat is necessary to raise the temperature of the calorimeter a given amount—i. e., its hydrothermal equivalent.

The temperature of the copper wall is determined by means of an electric-resistance thermometer somewhat like that described on page 313 for determining the temperature of the air. In this thermometer, however, each of the six coils of nickel resistance wire is wound on a thin fiber strip about 12 cm. long and 1 cm. wide, and is covered with a thin layer of silk and lacquered, the completed bulb being about 1.5 mm. thick. A strip of brass, slid into guides soldered to the copper wall, presses each coil firmly against the wall so that there is close thermal contact with metal on each side of the coil; hence, changes in the temperature of the wall affect the resistance wire very quickly. These six coils, joined in series by well-insulated No. 16 copper wire, are distributed on the side walls and ceiling in such manner as to show the average temperature of the total mass of copper. The terminals of the series of coils are connected with the special switch, mentioned on page 334, and through that with the temperature indicator (Wheatstone bridge) on the observer's table. The bridge and galvanometer are sensitive to resistance changes

in the thermometer coils that would result from a change of 0.01 degree in the temperature of the copper wall.

The hydrothermal equivalent of the calorimeter has been estimated from determinations of the quantity of heat that had to be dissipated in the chamber to raise the temperature of the copper wall 1 degree, and the amount of heat that was imparted to the air of the chamber when the temperature of the copper wall fell 1 degree, while the thermal conditions of the zinc walls were kept in equilibrium with those of the copper wall during the change. The capacity for heat as determined in both ways was not far from 40 Calories. From the weights and specific heats of the materials entering into the construction of the chamber the hydrothermal equivalent was calculated to be between 35 and 40 Calories. According to these figures, the quantity of heat in the chamber should be increased by 40 Calories with a fall of 1 degree, or decreased by 40 Calories with a rise of 1 degree in the temperature of the copper wall, if the thermal conditions of the zinc wall were in equilibrium with those of the copper wall while the change occurred.

This will be the case, provided the change in thermal conditions has occurred in such manner as to affect the iron supporting structure the same as the copper wall. In constructing the calorimeter no provision was made for determining the actual temperature of the structure, the assumption being that the thermal conditions of the iron framework would also be controlled by the regulation of those of the zinc wall, so that the temperature of the iron would be quickly brought to that of the copper wall and would vary with it. Experience has shown, however, that in some circumstances the change in thermal conditions of the iron may lag somewhat behind that of the copper wall; hence, it is much more desirable to keep the temperature of the walls of the chamber as constant as possible for the whole length of an experimental period than to depend upon the correction for change in temperature. With a sudden change in the rate of dissipation or absorption of heat in the chamber near the close of a period, which would affect the temperature of the copper wall, there might be an error in the measurement of heat for the period in spite of the allowance for temperature change. (See p. 346.)

Change in Body Temperature of the Subject of an Experiment

When the human body is the source of heat in the chamber, allowance must be made for the heat involved in any change in its temperature, as the body has a considerable thermal capacity. From the best available data it would appear that a change of 1 degree in the temperature of the body involves a change of 0.83 Calorie in the quantity of the heat accumulated for each kilogram of body weight. A rise in body temperature would mean that the store of heat in the body has been increased a certain amount, which would have to be added to that eliminated by the body and measured by the calorimeter during the period in which the rise

occurred in computing the quantity of heat actually produced by the body in the period. Conversely, a decrease in body temperature would mean that a certain amount of the heat that had accumulated in the body previous to the experimental period had been eliminated with that produced by the body during the period and should be subtracted from the quantity measured by the calorimeter in determining the quantity actually produced in the period.

The weight of the body can be ascertained accurately. The specific heat assumed is an estimate, but is probably fairly accurate. The temperature of the body as a whole can not be determined precisely, because it is not the same in all parts of the body. The temperature at the surface is noticeably lower than that of the interior, and that of the tissue in one region differs from that of the tissue in another. It seems probable, however, that, except perhaps at the surface, a change in temperature in one part of the body is accompanied by a corresponding change in the others; hence, the amount of temperature change, which is the factor concerned in the correction here considered, may be ascertained with a fair degree of accuracy from measurement of temperature where possible, but preferably below the surface.

By means of an electric-resistance thermometer the temperature of the subject in the chamber, at the spot at which the thermometer is located, may be ascertained at any given moment by the observer outside. A coil of wire of variable resistance, mounted so that it may be worn by the subject and kept at the temperature of the body, is connected with a Wheatstone bridge on the observer's table, by which the variations in resistance of the coil, due to changes in body temperature, may be observed, connection between the bridge and the thermometer coil being made through the special switch mentioned on page 334.

One type of thermometer bulb, designed for use in the rectum, is a coil of platinum wire having a resistance of 20 ohms at 37° C., inclosed in a thin steel shell or capsule 5 cm. in length and 5 mm. in external diameter. Since this thermometer may be kept in place for considerable periods without discomfort, a virtually continuous record of body temperature may be obtained, depending upon the frequency of the readings by the observer, and fluctuations may be followed for long or short periods as desired, but the temperatures at the beginning and end of the experimental period are the ones essential for the correction here considered. In another type, designed for measuring temperature of the body surface, the wire is wound in a flat spiral coil 15 mm. in diameter, mounted in a frame of thin, hard rubber by which it may be held against the skin. This coil rapidly acquires the temperature of the skin.

In some cases the temperature is measured by means of accurate clinical thermometers, inserted by the subject under the tongue or in the axilla, which are afterward read by the observer.

Heat from Other Sources

The store of heat in other objects in the chamber than the body of the subject—e. g., furniture—is increased by a rise and decreased by a fall in the temperature of the air surrounding them, and allowance must be made for the effect of such change in their condition upon the measurement of the quantity of heat produced in the chamber. The quantity of heat involved is computed from the weight, the specific heat, and the change in the temperature of the objects. The latter factor is not definitely known, however, as no provision is made for actually measuring the temperature of such objects; the assumption being that their change in temperature will be the same as that of the air, which is determined. Where the change occurs slowly, any error involved in such assumption is probably negligible; but this is not true when any considerable change occurs in a short period. This is another reason for keeping the temperature of the air of the chamber as constant as possible.

Allowance must be made also for gain or loss of heat due to the introduction of objects into the chamber at a temperature above or below that of the air. Hot food or drink, for example, admitted through the food aperture would add heat to that produced in the chamber, while cold material would absorb some of the heat produced. The temperature at which any material is admitted is recorded, together with its weight and character, and from these data, with the specific heats of the various articles, the necessary corrections are computed.

The electric fan by which the air of the chamber is agitated and the electric light, when one is used, both generate heat which forms part of that measured by the calorimeter and for which allowance must be made. The quantity of heat produced is computed according to the formula $\frac{EIt}{4.183}$ = small calories at 20° C., E being the voltage and I the amperage of the current in the lamp and the fan, and t the time in seconds during which it was used.

The divisor, 4.183, is the number of international joules (watt seconds) equivalent to one small calorie at 20° C. (10, p. 255). The lamp and fan are connected in such manner that the voltage and amperage of both may be determined at the same time by calibrated measuring instruments on the observer's table, the readings of which are recorded at regular intervals. That the heat may be generated at a uniform rate, the current is taken from a generator which has an automatic regulator to keep the voltage constant within quite narrow limits.

APPARATUS FOR MEASURING MUSCULAR WORK PERFORMED BY THE SUBJECT OF AN EXPERIMENT

For the study of many problems involving the performance of muscular work some method of measuring the amount of work done is requisite. An apparatus (9, p. 48; 8, p. 11) that was devised in connection with the nutrition investigations of the Department has proved very

successful for measuring work done with the muscles of the legs. The principle of the device is that of the electric brake. It is designated a "bicycle ergometer," since it bears some resemblance to a bicycle; in fact, in its construction all of a bicycle except the wheels was used, and the work done in operating it is of the same kind as that involved in propelling a bicycle. In the ergometer, however, the front wheel of the bicycle is replaced by a pedestal and the rear part of the frame is supported by a rack, so that a heavy copper disk, 40.5 cm. in diameter and approximately 6 mm. in thickness, which replaces the rear wheel of the bicycle, will rotate freely on its ball-bearing axis. An electromagnet is attached to the frame, with its poles on opposite sides of the disk, with the inner edge of the rectangular-pole faces coincident with the circumference of the disk, and with the face of each pole 1 mm. from the surface of the side of the disk. When there is no current in the field coils of the magnet, the amount of energy required to cause the disk to rotate between the pole faces is small, being merely that necessary to overcome the friction of the bearings and the resistance of the air against the moving parts; but when there is a current in the coils, with its resulting magnetization, currents are induced in the disk rotating in the magnetic field, which tend to prevent it from rotating. The brake effect depends upon the flux density of the field, which varies with the strength of the magnetizing current. The amount of work done by the subject on the ergometer is therefore easily controlled by adjusting a rheostat in series with the coils of the magnet until the strength of the current is that which will result in the desired resistance to be overcome in causing the disk to rotate. A particular advantage in this apparatus is the constancy and uniformity with which the effect may be reproduced.

The power applied to the pedals when work is done on this ergometer is converted into heat, a small part of it by the friction of the moving posts of the mechanism and the rest by the energy transformations in the disk. The quantity of heat thus produced varies with the intensity of the magnetic field and also with the rate of rotation of the disk. From calibration of the ergometer in the calorimeter the amount of heat produced by each rotation of the disk in the magnetic field was determined for a considerable variety of conditions of velocity of the disk and strength of magnetizing current within the range commonly employed in experiments. By use of curves derived from the data of calibration the heat equivalent of the external muscular work performed by the subject on the bicycle ergometer is computed directly from the total number of rotations of the disk, as shown by an automatic counter, and of strength of current in the field coils, as shown by an accurate ammeter.

The heat produced in the ergometer is measured by means of the calorimeter, together with that eliminated from the body; but since the former can be ascertained, as just explained, it may be subtracted from the total heat measurement, when the amount of heat produced by the subject in performing muscular work is computed.

TESTS OF THE ACCURACY OF THE RESPIRATION CALORIMETER

At frequent intervals the accuracy with which the respiration calorimeter will function is tested by burning ethyl alcohol in the chamber in such manner as to insure complete combustion and measuring with the apparatus the amounts of oxygen consumed and of carbon dioxid, water vapor, and heat produced. Commercial alcohol, pure in quality and containing about 90 per cent of ethyl hydroxid, is satisfactory for the purpose. The actual percentage is ascertained from determination of the specific gravity of the alcohol. The amount of oxygen that would be required to burn 1 gm. of the commercial alcohol, and the amounts of water vapor and carbon dioxid that would result from the combustion, are computed from the chemical equation for the reactions occurring in the combustion of ethyl hydroxid, with allowance for the water present in the sample burned. The heat of combustion of ethyl hydroxid at constant pressure is taken as 7.08 Calories per gram.

The burner used in these experiments was made of two concentric tubes of thin brass 5 cm. in length, the outer tube being 18 mm. in external diameter. At the lower end each tube is soldered to a brass ring, which provides an annular space between them 3 mm. wide, in which is a wick of asbestos; and as the inner tube is open at both ends, there is a center draft for the flame. No products of incomplete combustion have been found in the air of the chamber during a test in which alcohol was burned with this burner.

The burner is soldered to one end of a piece of 4-mm. copper tubing, the other end of which passes through the wall of the chamber to the alcohol supply outside, from which the burner is fed in such manner that the rate of consumption is uniform. Attached to the outer end of the copper tube is an elbow of 4-mm. glass tubing, with a side outlet in the vertical arm to provide for an overflow. The height at which this outlet is set with relation to the top of the burner governs the rate of consumption of the alcohol. The level having been fixed, alcohol is fed into the vertical tube so that some of it will drop regularly from the outlet. The rate of combustion in the chamber is then very constant. The overflow alcohol is caught in a small bottle, which is weighed with the supply flask at the end of each period, the loss in weight of both containers showing the quantity of alcohol burned. The connection between the overflow bottle and the outlet and also that between the supply flask and the feed tube are such that the loss of alcohol by evaporation is negligible.

The results of alcohol check tests of the respiration calorimeter indicate that the determinations made with it are at least sufficiently accurate for investigations of the character in which it is used. This is shown by the data in Table II, which summarizes the results of two tests selected from many equally good.

In November, 1913, in a 3-hour period, 56.3 gm. of commercial alcohol containing 88.32 per cent of ethyl hydroxid were burned to test the

accuracy of the determinations when heat was produced in the chamber at a rate of about 120 calories per hour. For all four factors the quantities determined were slightly larger than those computed from the composition of the alcohol, the discrepancies amounting to 1.5 per cent for oxygen, 0.7 per cent for water, and the same for heat, and 0.2 per cent for carbon dioxid. The respiratory quotient—i. e., the ratio of the volume of carbon dioxid produced to that of oxygen consumed in the combustion of alcohol—is 0.667; in the test the ratio of the values found was 0.658. Similarly, the ratio of the number of Calories of heat produced to the number of grams of carbon dioxid produced is theoretically 3.705, whereas in the test it was 3.725.

TABLE II.—*Data obtained in the combustion of alcohol in the respiration calorimeter*

Date.	Oxygen.		Water.		Carbon dioxid.		Heat.		Quotients.	
	Found.	Required.	Found.	Required.	Found.	Required.	Found.	Required.	Respiratory (CO_2: O_2).	Thermal (Cal.: CO_2).
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Cal.*	*Cal.*		
Nov. 21, 1913...	105. 1	103. 6	65. 4	64. 9	95. 1	94. 9	354. 2	351. 7	0. 658	3. 725
Feb. 18, 1915...	139. 0	140. 7	97. 3	98. 5	142. 5	143. 9	535. 8	533. 3	. 669	3. 759

In February, 1915, in a 6-hour period, 85.35 gm. of commercial alcohol containing 88.25 per cent of ethyl hydroxid were burned, the rate of production of heat being about 90 Calories per hour. In this test the heat measured by the calorimeter was nearly 0.5 per cent greater than that computed, whereas the quantities of oxygen, water, and carbon dioxid measured were 1 to 1.3 per cent lower than those computed.

Another test made at frequent intervals provides a check on the accuracy of the calorimetric function of the apparatus. Electric energy is converted into heat within the chamber, and the amount produced in a given period, which can be computed very accurately, is compared with that measured by the calorimeter during the period. The electric energy is converted into heat in a coil of resistance wire suspended in the chamber. The amount of energy that is dissipated is computed from the values for the voltage and amperage of the current in the coil, the time in seconds, and the factor for converting watt seconds to small calories at 20° C., as explained on p. 342.

The resistance of the heating coil depends upon the desired heat production, the majority of the tests having been made with a coil having a resistance of 440 ohms, which allows 0.5 ampere of current to flow with a fall of potential of 220 volts. The resultant heat is approximately 95 Calories per hour, or about that produced by an average man sitting still. That the rate of production of heat may be constant, the voltage of the current is controlled by an automatic regulator; but the actual fall in potential is measured by a voltmeter connected to the terminals

of the coil, and the amperage of the current is measured by a milliammeter in series with it, the readings of both meters being recorded every minute, or oftener.

In a 2-hour test in January, 1915, the total heat production was 139.00 Calories, while the quantity measured with the calorimeter was 139.04 Calories. Such absolute agreement is not to be expected invariably, though with the conditions ordinarily prevailing in an electric check the two values should not differ by as much as 1 per cent. A discrepancy of that size would indicate need of attention to some part of the apparatus, or lack of attention to some details of operation. For example, in a 3-hour test in February, 1915, the total quantity of heat generated in the chamber was 370.19 Calories and that measured by the calorimeter was 377.39 Calories. The discrepancy between the two values was due to a considerable decrease in the temperature of the walls of the chamber during the last half of the first hour, resulting from a lowering of the temperature of the water entering the heat absorber. In the two hours following that the heat production was 245.87 Calories, as computed, and 246.75 Calories, as measured, a discrepancy of less than 0.4 per cent. The results in the first hour illustrate the statement made on page 340 regarding error in heat measurement when the temperature of the copper wall changes so quickly that its thermal conditions differ from those of the iron structure to which the wall is attached.

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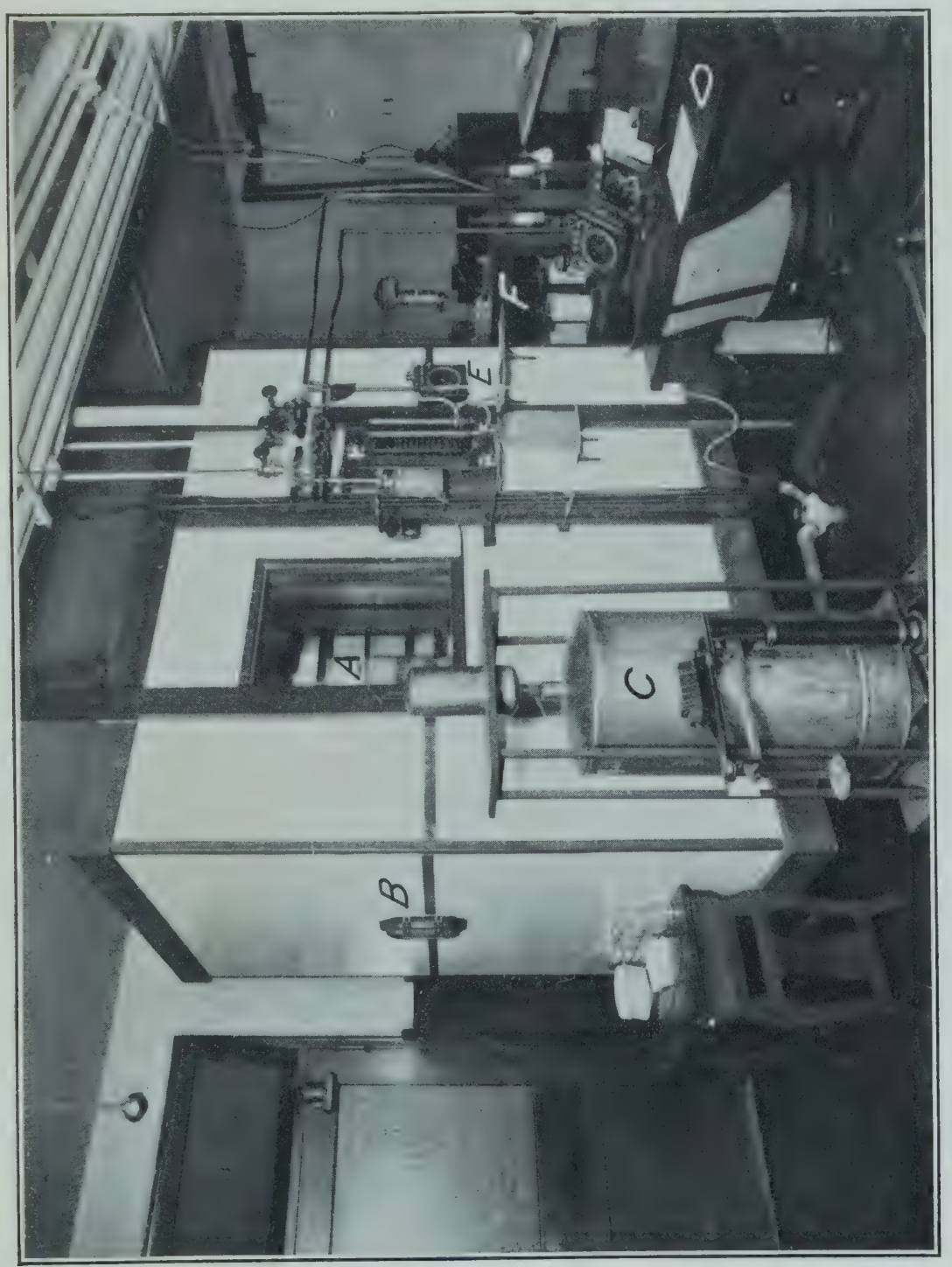
PLATE XXX

General view of the respiration calorimeter: *A*, Opening serving as door and window to chamber. *B*, Food aperture. *C*, Tank to catch water coming from the heat absorber in the chamber. *D*, Observer's table, with devices for measuring and regulating temperatures. Other temperature measuring and regulating apparatus pertaining to the calorimeter are not shown in this view. *E*, Thin rubber bag, resting on shelf, to serve as air tension equalizer. *F*, Table on which are motor and blower for maintaining circulation of air through chamber, and absorbers for purifying the air.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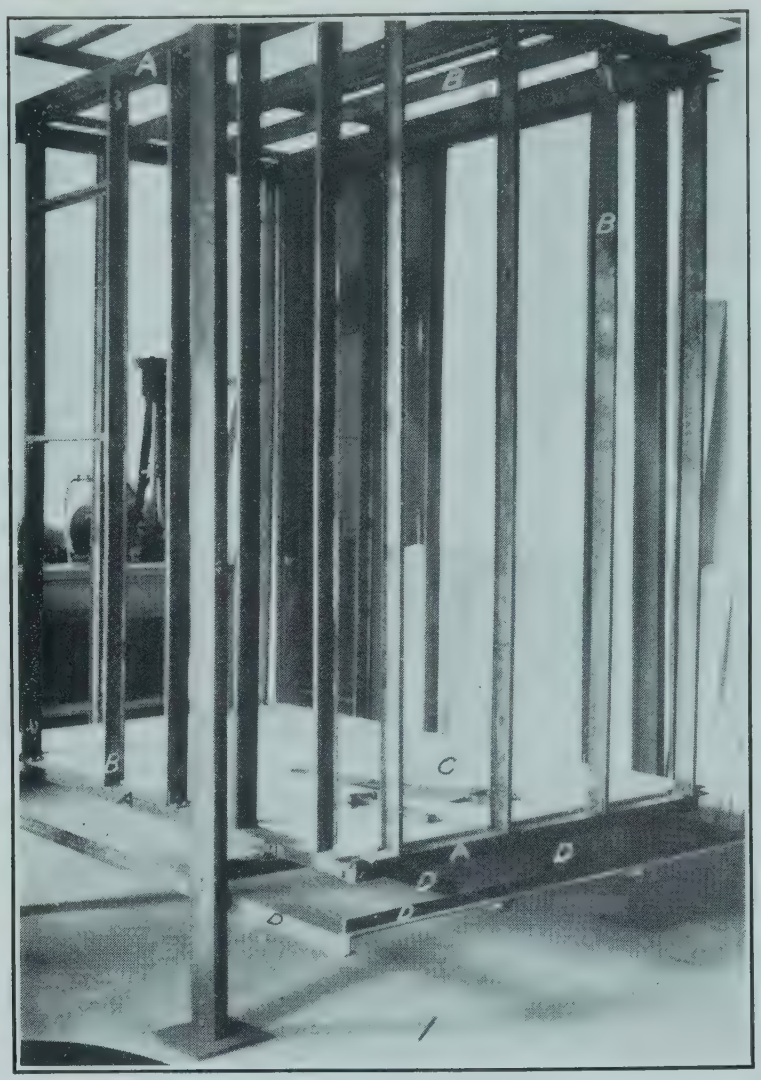
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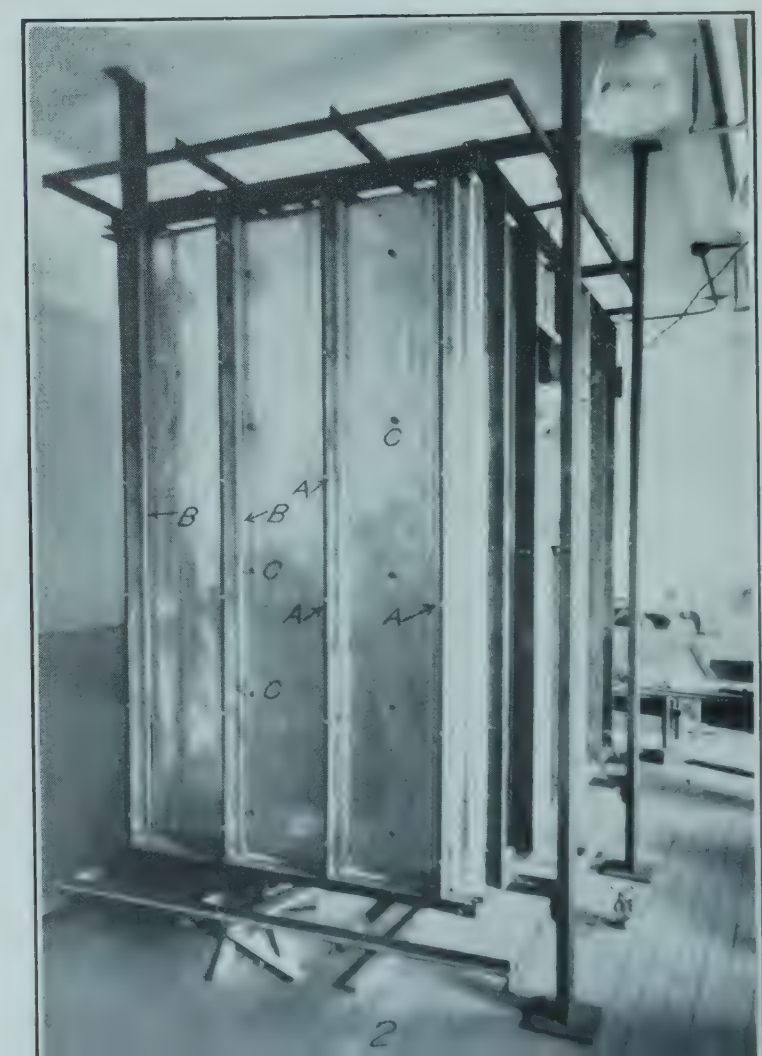
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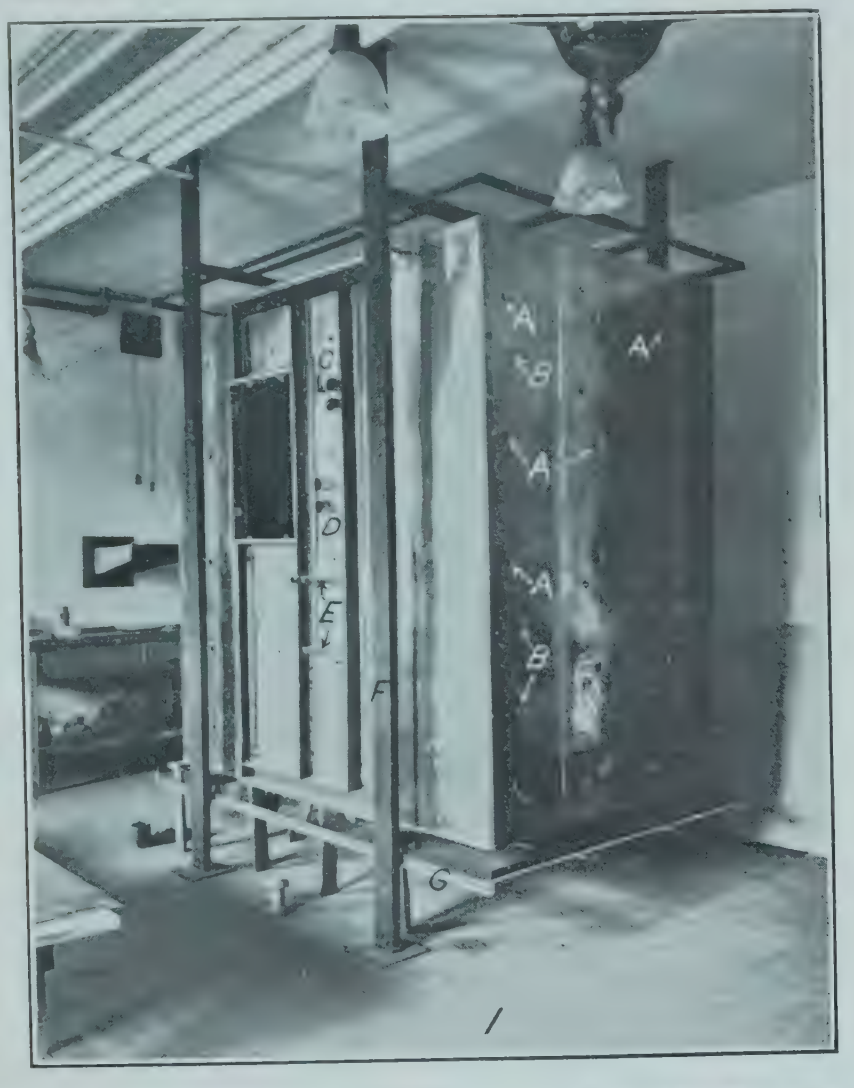

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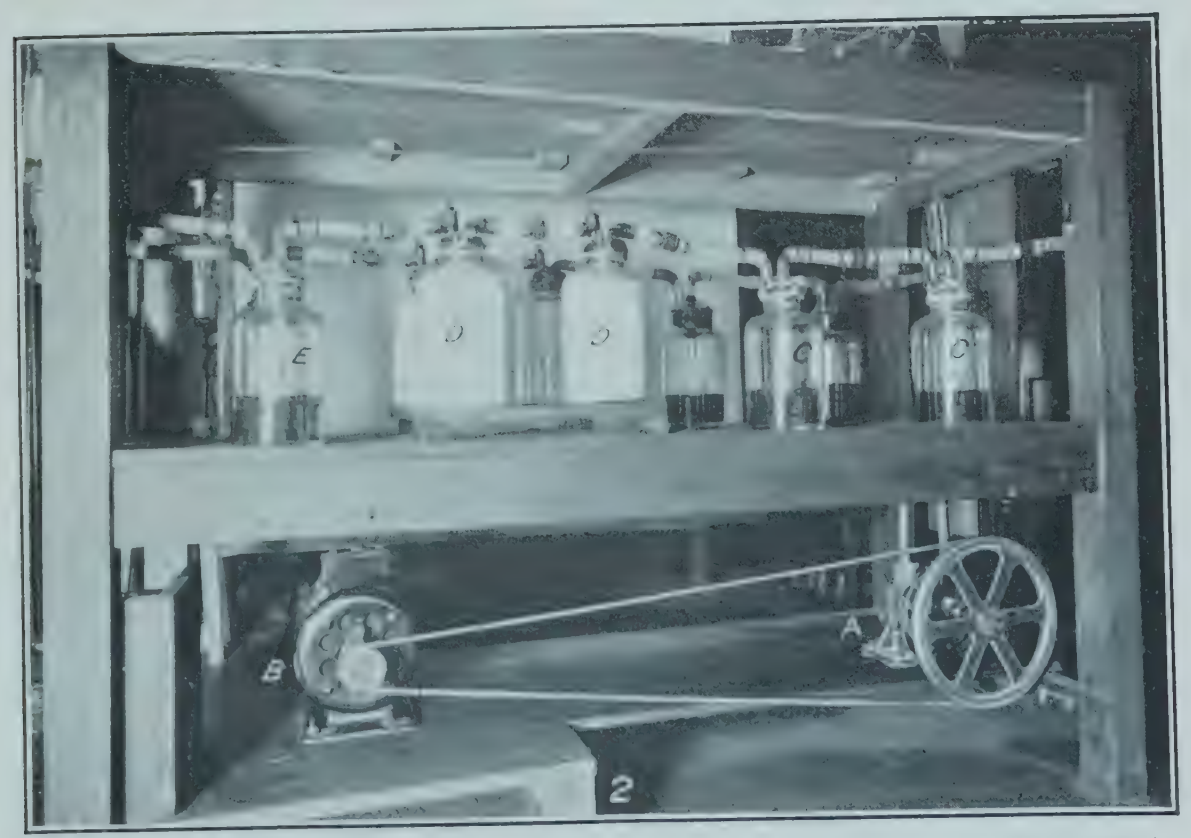

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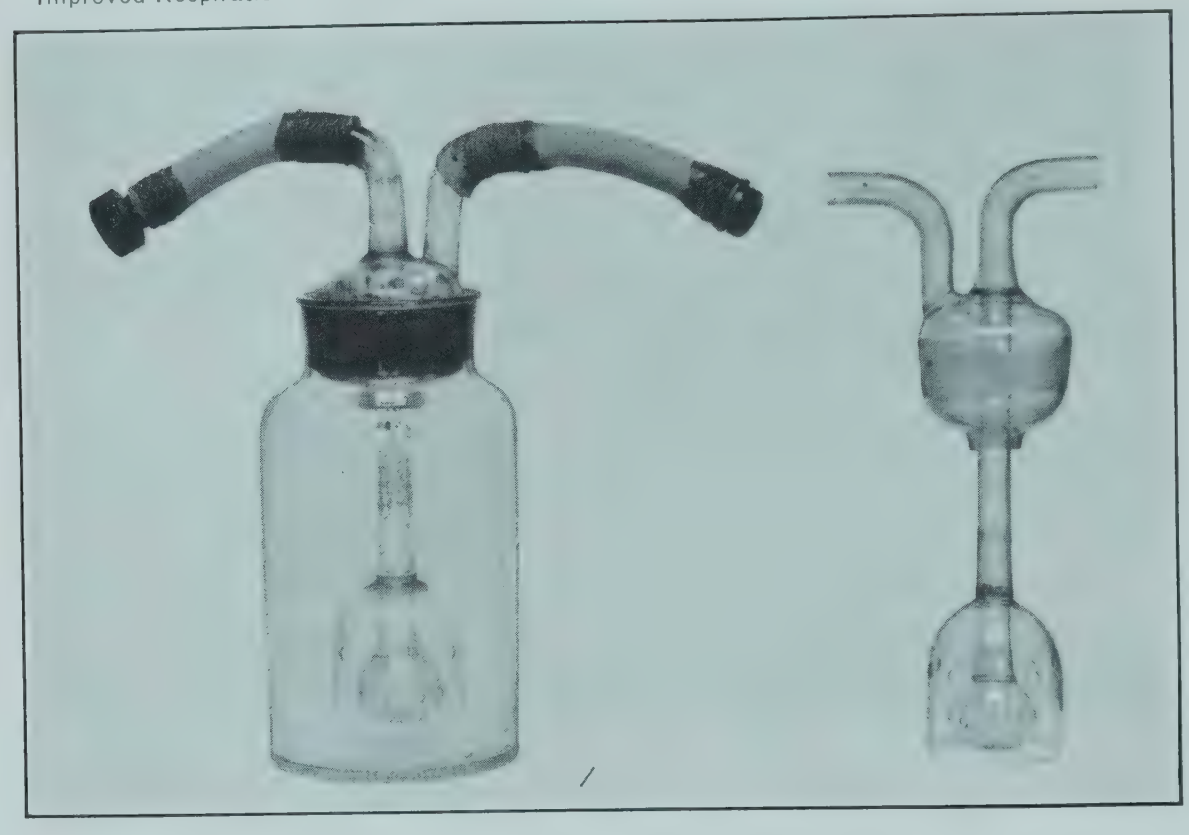

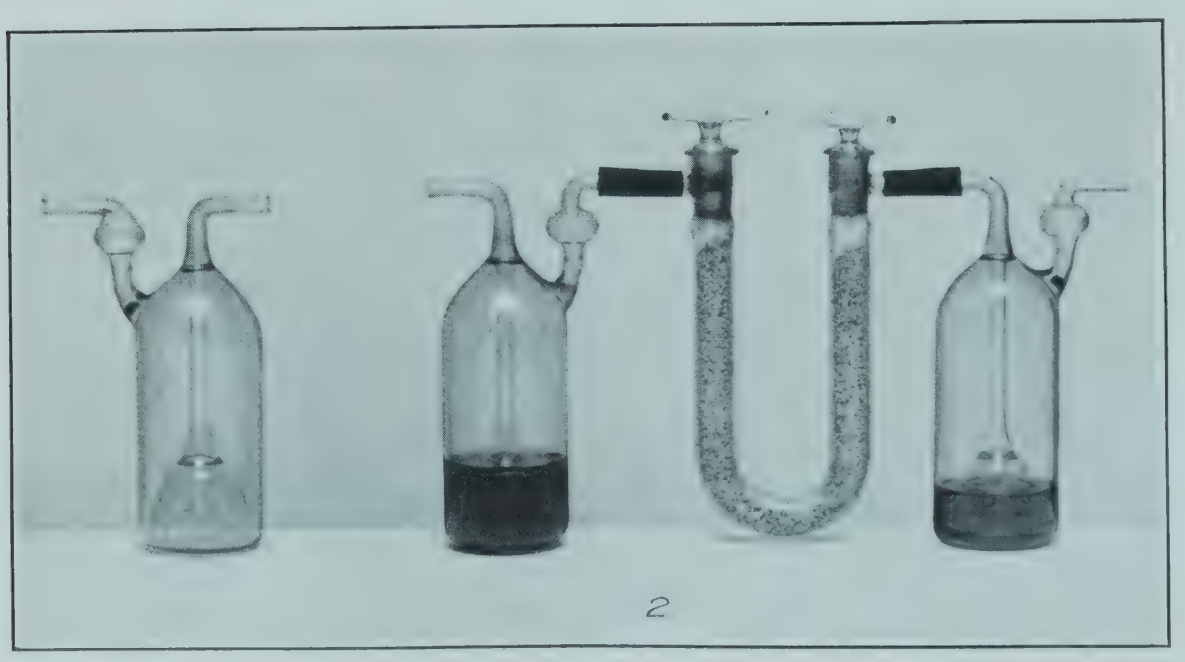

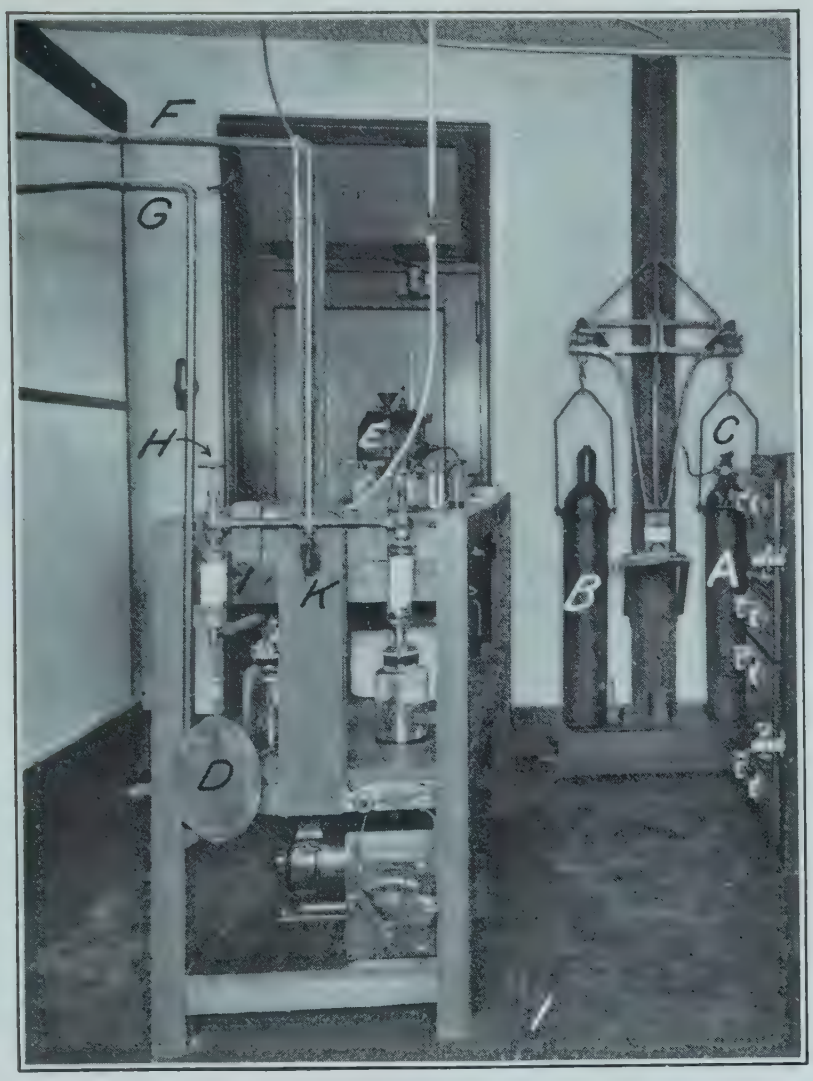
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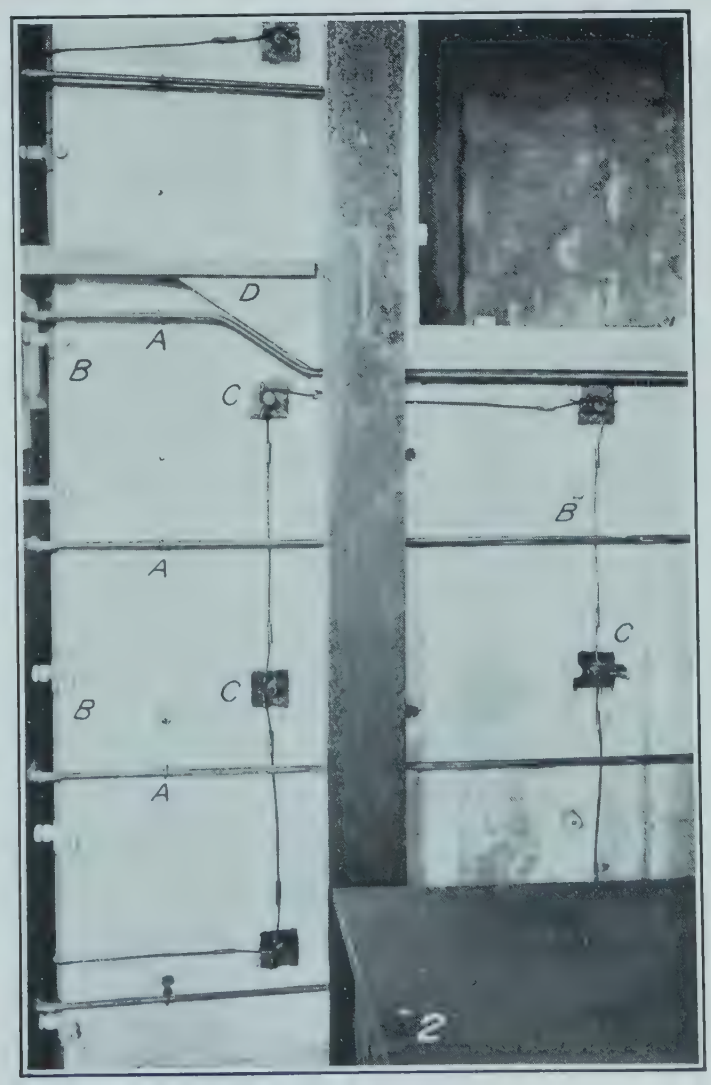
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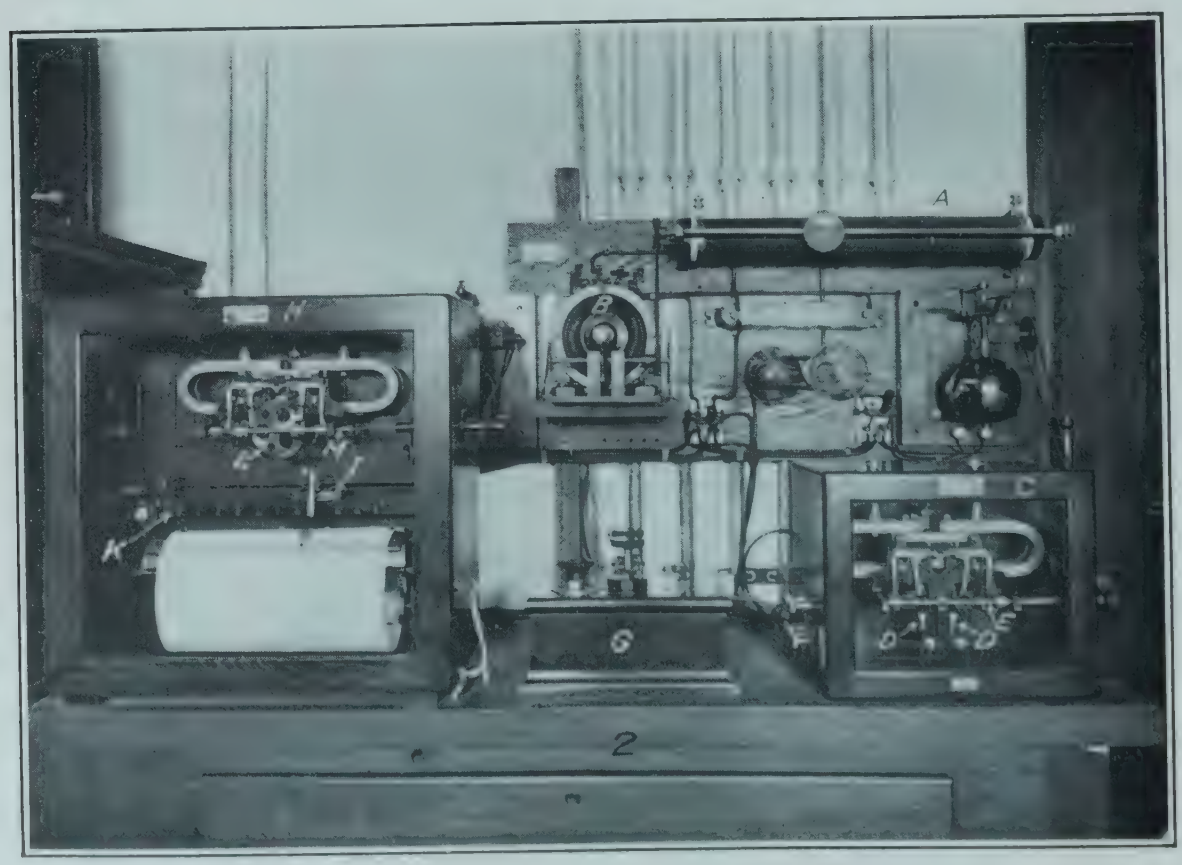
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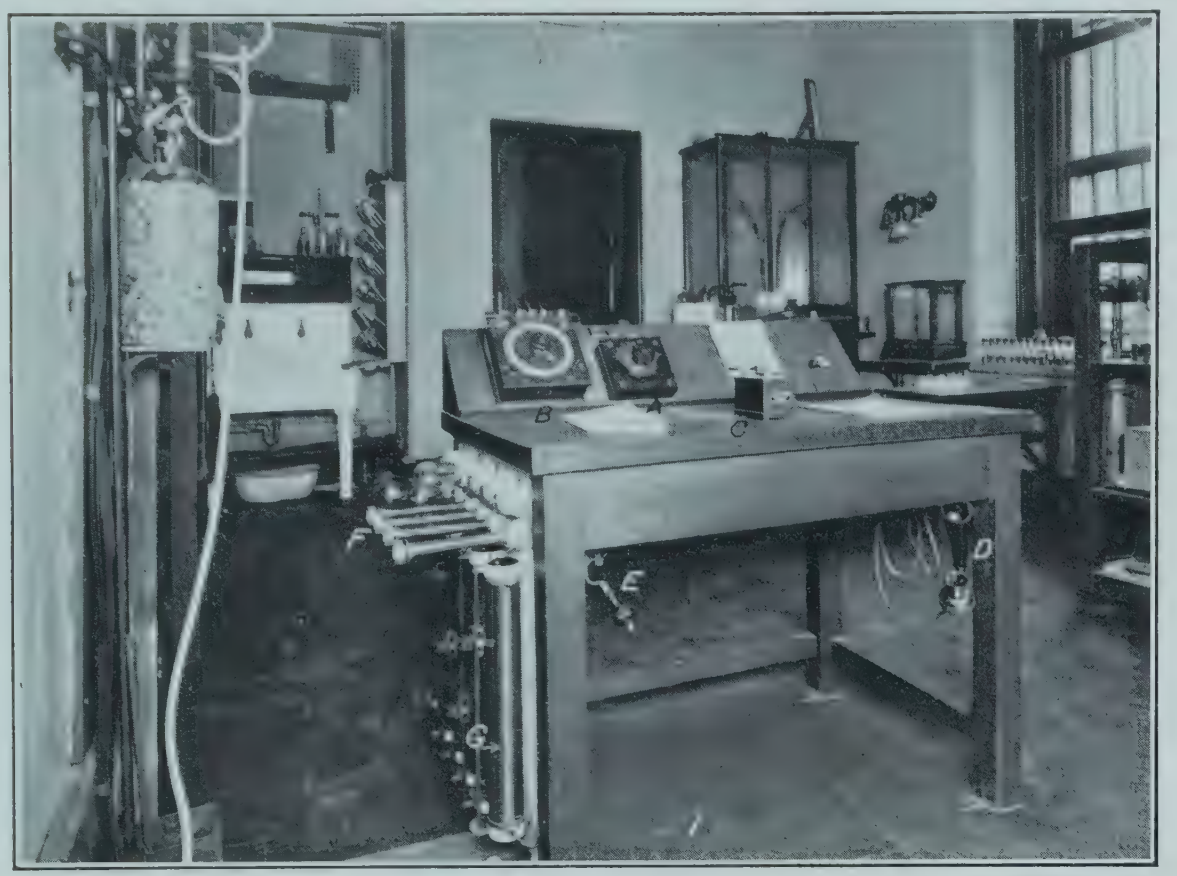
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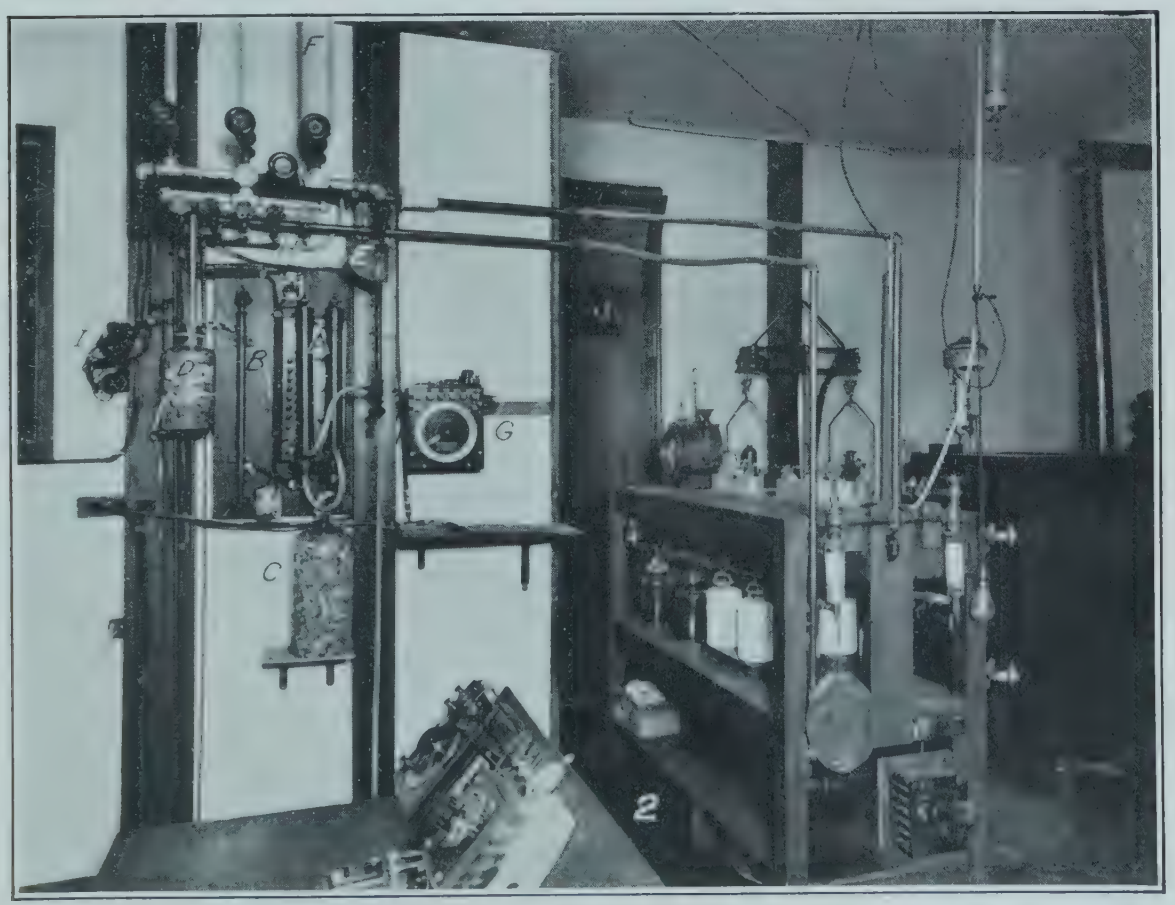
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OCCURRENCE OF MANGANESE IN WHEAT

By WILLIAM P. HEADDEN,
Chemist, Colorado Agricultural Experiment Station

The presence of manganese in various plants has been observed repeatedly. It is now stated that it has been shown to be present and has been determined in a great many grains, roots, leaves, and whole plants and that it is probably present in all plants.

It is generally asserted that this manganese is to be considered as an accidental constituent and that it has no physiological function. An opposite view, however, is held by some who maintain that it performs an important catalytic function in the plant. Bertrand, for instance (1),[1] has shown that the enzym laccase can not act as an intermediary between the oxygen of the atmosphere and certain organic compounds in the cells of the plants if it is deprived of its manganous oxid, with which it forms a feeble compound. He has further shown that this enzym, laccase, is very generally diffused throughout the vegetable kingdom and that whenever laccase functions in the nutrition and growth of plants, manganese is a necessity. The amount of manganese contained in this laccase is only 0.12 per cent.

There are some data on the effects of manganese on wheat, oats, barley, grass, etc. Guthrie and Cohen (3) attribute the death of grass on certain spots to the presence of 0.254 per cent of manganic oxid in the soil. They account for the death of barley in certain soils in the same manner. Voelcker (7), experimenting with different salts of manganese, applied in quantities up to 200 pounds per acre, obtained results which are summarized as follows for experiments with wheat:

> The chlorid and nitrate produced a good color in the plants. The iodid distinctly retarded germination and growth. The untreated plots produced as good plants as any of the others except those which had received an application of nitrate or phosphate. The phosphate, chlorid, sulphate, and red oxid (Mn_3O_4) each gave an increase in yield.

Kelley (6) states that some plants vegetate normally in the presence of manganese salts, others are stunted in growth and die back from the tops of the leaves, which turn yellow or brown and sometimes fall off. Plasmolysis is produced. Chlorophyll may be destroyed as in the pineapple. The ash shows an increased percentage of manganese. The percentage of lime is increased and that of the magnesia is decreased. This ratio, on the authority of Loew, is considered an important one. The absorption of phosphoric acid is lessened. The formation of manganous phosphate is suggested as the possible cause of this.

[1] Reference is made by number to "Literature cited," p. 355.

According to Kelley, the action of manganese, especially if present in the soil in relatively large quantities, 2.34 to 9.74 per cent, produces very radical changes in the nutrition of the plant (5). But such quantities of manganese as correspond to these percentages are not often found in soils.

Brenchley (2, p. 583) sums up her observations on the effects of manganese on barley thus:

> Manganese sulfate, though not an actual toxic to barley, retards the growth very considerably if supplied in moderate quantities. Minute traces of the salt have a decided stimulative action both on the root and shoot. * * * When supplied in sufficient concentration manganese is taken up by the plant and deposited in the lower leaves.

Jost (4, p. 87), in treating of the nonessential ash constituents absorbed from the soil by plants, says of silica:

> Although silica may be quite superfluous from the chemical point of view, it may be of great service to the plant in the biological sense. Our knowledge of these subjects, despite the amount of work which has been expended on them, is still very imperfect, and it is possible to defend the assertion that all the ash constituents have definite functions to perform, although these have not as yet been determined in all cases, and although these constituents can not be considered as taking part in metabolic changes. * * * The occurrence of manganese may, however, be specially noted, as leading to the consideration of a new series of phenomena. It is not widely distributed in the earth, and yet is found, though only in traces, in very many plants.

In discussing nutritive and stimulative materials he uses the following language concerning iron (4, p. 88):

> This distinction is not readily made out in all cases; iron, for example, is a difficult element to deal with, because it is essential only in the minutest traces, and is possibly both a nutrient and a stimulant.

Iron is definitely recognized as essential for the growth of plants, though the quantities required are exceedingly small.

The presence of manganese in wheat straw has been mentioned by others, but nowhere have I found its quantity given, and it is not mentioned in connection with the grain. The statement of M. Bertrand (1) that "manganese has been found in many grains"[1] is the only one known to me that may indicate the occurrence of this element in the wheat kernel.

In examining the mineral constituents of wheat (*Triticum* spp.) I was struck by the fact that there was uniformly enough manganese present to come down with the calcic oxalate and to impart a decided brown color to the calcic oxid when ignited. A few preliminary determinations revealed the fact that there was as much or more manganese than iron present. At the time this observation was made I had examined 25 samples of wheat and had found manganese present in every sample. These samples had been grown on the same soil, though the different plots had not received the same fertilization. The supply of manganese

[1] Author's translation.

in the soil is about 0.10 per cent, calculated as elemental manganese. If the manganese be an accidental constituent, as is usually held to be the case, its presence must be due to the supply in our soil, but the amount taken up appears to be very nearly constant, irrespective of the soil.

In order to ascertain whether the manganese is universally present in the wheat kernel and to determine in what quantity it is usually present, I obtained samples of wheat from a number of localities in the United States and Canada and from three European countries. While manganese is probably present in every cultivated soil, it is very rarely the case that it constitutes more than a small fraction of 1 per cent, while iron is usually present in much more considerable quantities. The amount of manganese present in the soil bears no relation to that of the iron. In the soil on which our wheat samples were grown, the metallic iron found by a mass analysis of the soil was a trifle over 30 times as great as the total amount of manganese. The analytical results given subsequently show that this is not the ratio in which the two elements are present in the kernels and not even in the green plant or in the ripe straw. It does not seem probable that the manganese has been absorbed simply because it existed in the soil associated with iron, if this indeed be the case in any strict sense, for the association might be with calcium as well as with iron.

The method used in determining manganese in grain was to take 10 gm. of ground, air-dried wheat, dissolve it in concentrated nitric acid, and evaporate the solution to a thick, gummy, brown mass. This was then heated over a free flame till all volatile matter was expelled. The dish was then placed in a muffle and most of the carbon burned off. After removal and cooling, a few (4 or 5) cubic centimeters of concentrated sulphuric acid were added. The sides of the dish were washed down with a little water and the solution evaporated at last on a sand bath till vapors of sulphuric acid escaped freely. After cooling, this was taken up with water, boiled, and filtered into a 200 c. c. flask. The residue on the filter was burned, taken up with a little sulphuric acid as before, and the solution filtered and added to the first filtrate. The combined filtrates should be about 150 c. c. in volume and contain about 5 per cent of sulphuric acid. A little silver sulphate (from 25 to 30 mgm.) was next added and then 4 or 5 gm. of ammonic persulphate. The solution was placed on a boiling water bath and allowed to stand as long as the color deepened. It was then cooled, made up to volume, and compared with the standard, which had been prepared in the same way. All reagents should be tested by making a blank.

Manganese in the straw was determined in the same way, except that the silica was removed by evaporating in a platinum dish with the addition of hydrofluoric acid.

In Tables I and II are given the variety, the fertilizer applied, and the percentage of iron and manganese found in wheat from Colorado and

Idaho. Table III gives the percentage of manganese only in wheat from different regions, while Table IV gives the percentage of manganese found in various other grains.

TABLE I.—*Iron and manganese in Colorado wheat*

Variety.	Fertilizer per acre or other treatment.	Iron.	Manganese.
		Per cent.	*Per cent.*
Defiance	80 pounds of nitrogen	0.005	0.005
Do	40 pounds of phosphorus	.005	.004
Do	150 pounds of potassium	.003	.004
Do	None	.003	.004
Red Fife	80 pounds of nitrogen	.004	.004
Do	40 pounds of phosphorus	.004	.004
Do	150 pounds of potassium	.004	.005
Do	None	.005	.005
Kubanka	80 pounds of nitrogen	.004	.005
Do	40 pounds of phosphorus	.003	.004
Do	150 pounds of potassium	.003	.005
Do	None	.004	.005
Defiance	Fallowed 1 year	.006	.005
Marquis	do	.005	.005
Red Fife	do	.005	.007
Kubanka [a]		.003	.003
Kubanka [b]		.004	.004

[a] Yellow berry; soft. [b] Flinty; hard.

TABLE II.—*Iron and manganese in Idaho wheat* [a]

Variety.	Fertilizer per acre.	Water per acre.	Iron.	Manganese.
		Feet.	*Per cent.*	*Per cent.*
Marquis	None	1	0.006	0.006
Do	do	2	.005	.006
Do	do	3	.007	.005
Do	16 loads of manure	1	.006	.006
Do	do	2	.006	.005
Do	do	3	.006	.005

[a] I am indebted to Mr. Don. H. Bark, of Boise, for these samples.

TABLE III.—*Manganese in wheat from different localities* [a]

Variety.	Locality.	Per cent.	Variety.	Locality.	Per cent.
Poole	Missouri	0.007	Huron	Province of Quebec, Canada.	0.006
Do	Ohio	.006			
Jones's Longberry.	Pennsylvania	.006	Kubanka	Petrograd, Russia.	.004
Iowa Red	Kansas	.005			
Mealy	New York	.008	Bore	Svalof, Sweden	.004
Red Fife	Province of Manitoba, Canada.	.006	Wilhelmina	Holland	.004

[a] I am under obligation to the respective officers of the various experiment stations for the samples of American and Canadian wheats and to the Bureau of Plant Industry, United States Department of Agriculture, for the samples of foreign wheats.

TABLE IV.—*Manganese in other grains*

Variety.	Locality.	Per cent.
Emmer (*Triticum dicoccum*)	Fort Collins, Colo.	0. 004
Rye (*Secale cereale*)	do	. 004
Barley, bald (*Hordeum* sp.)	do	. 002
Oats (*Avena sativa*)	do	. 005

Several samples of corn (*Zea mays*) were tested, a large white variety (Meerschaum) from Missouri, a yellow variety, irrigated, from Grand Junction, Colo., a yellow variety, not irrigated, from Akron, Colo., and a white variety, irrigated, from Fort Collins, Colo. These samples contained so minute a trace of manganese that it could be detected only with great difficulty when 10 gm. of the grain were used for the test.

In addition to the determinations of manganese given in the preceding tables, I have found it uniformly present in the ash of Colorado wheats and also in wheats from California, Nevada, Washington, Montana, South Dakota, Minnesota, Kentucky, and Tennessee. It can, I believe, be accepted as being universally present in the wheat kernel and likewise in the wheat plant, but it is not as abundant in the dried plant as in the kernels. The ratio of the iron to manganese is higher in the plant. The risk of obtaining iron from dust, etc., in the case of the plant is, it is true, greater than in the case of the kernel, but I think that we are fairly safe in assuming that the iron found in our samples belongs to the plant constituents and is not derived from extraneous sources (Table V).

TABLE V.—*Iron and manganese in dried wheat plants*

Variety.	Date.	Fertilizer per acre.	Iron.	Manganese.
			Per cent.	*Per cent.*
Defiance	July 28, 1913	120 pounds of nitrogen	.....	0. 004
Do	do	60 pounds of phosphorus	0. 010	. 003
Do	do	200 pounds of potassium	. 010	. 003
Red Fife	do	60 pounds of phosphorus	. 010	. 004
Do	do	200 pounds of potassium	. 015	. 004
Kubanka	Aug. 6, 1914	60 pounds of phosphorus	. 008	. 002
Do	do	200 pounds of potassium	. 013	. 002

Of the preceding samples only the last two were ripe; the others were cut from 8 to 12 days before being ripe enough to harvest.

The iron present in the straws is from two and one-half to six times as great in amount as the manganese, while in the kernels the manganese is approximately equal to the iron and at the same time is higher, as a rule, than in the straw.

The iron was determined gravimetrically in every case and the manganese colorimetrically. The variation in the iron found is great if calculated on the minimum amount found; still the difference between the

minimum and maximum, in spite of the difficulties of the analysis, is only 0.004 per cent, calculated on the air-dried wheat. The quantity of manganese found shows about the same maximum variation, but the determinations are mostly quite uniform without regard to the State or country in which the wheat was grown.

The samples given represent great differences in cultural conditions of both climate and soil, and yet the manganese is always present and in approximately the same quantities; in fact, a greater regularity is found in this respect than for iron in the determinations made. Iron is accepted as an essential constituent of the plant, while the manganese is held to be a nonessential one by most writers.

Bertrand (1), however, has shown that manganous oxid is essential to the action of laccase; and further, that this enzym is universally present in plants and fulfills a definite function in their metabolism, from which he concludes that manganese is an essential mineral constituent of most, if not of all, plants.

The reaction shown when a fresh surface of a potato is treated with a tincture of guaiacum is attributed to the oxidizing action of laccase. If the statements of Bertrand be correct the potato should contain manganese. For this reason I determined the manganese in a potato, using a single tuber, and found the amount of manganese in this potato, which had been dried at 100° C. for 24 hours, to be 0.0003 per cent, corresponding to from 0.00005 to 0.00006 per cent of the fresh tuber. This quantity seems very small, but even much smaller quantities of manganese in nutritive solutions produce decided effects upon vegetation. Brenchley (2, p. 579), in discussing her experiments to determine the effects of manganese upon the growth of barley, says:

> At this date [11 weeks from the beginning of the experiment] it was evident that manganese was deposited in the leaves even at so low a concentration as 1:1,000,000 M. S. and in some cases traces could even be observed in 1:10,000,000 M. S.

The percentages given in my determinations are for elemental manganese; Brenchley used manganous sulphate with five molecules of water. She points out that the effects of manganese may be modified by the relative supply of nutrients.

SUMMARY

(1) Manganese seems to be present in wheat wherever grown, irrespective of the conditions of soil and climate.

(2) Manganese is present in the wheat kernel in about the same proportion as iron, though iron greatly predominates in soils.

(3) Fertilizers applied to the soil did not affect the amount of manganese stored in the kernels.

(4) Variation in the quantity of water applied, from 1 to 3 feet, did not affect the amount of manganese in the grain.

(5) I do not wish to draw conclusions from my facts relative to the essential character of manganese as a mineral constituent of plants, though these facts seem to support this view for wheat and possibly for emmer, rye, oats, etc. It seems improbable that a nonessential constituent would occur in all samples and in essentially the same quantity under such a variety of conditions.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., NOVEMBER 29, 1915 NO. 9

ASH COMPOSITION OF UPLAND RICE AT VARIOUS STAGES OF GROWTH

By P. L. GILE, *Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

INTRODUCTION

The following ash analyses of upland rice (*Oryza sativa*) at various stages of growth were made in connection with a study of the effect of lime-induced chlorosis on the ash composition of the plant. In the course of this work it was necessary to know particularly how the iron content of the plant varied with its age. The analyses are reported here, as it is believed that such data are of general importance in explaining certain peculiarities of crop growth.

Kelley and Thompson [1] have already investigated the composition of rice at different stages of growth, but their study did not suffice for our purpose, as it covered only the last half or third of the growing period and did not include iron and some other ash constituents.

EXPERIMENTAL METHODS EMPLOYED

The plants were grown in large cylinders sunk in the ground and protected by wire netting (4 meshes to the inch). Each cylinder afforded a surface of 7 square feet of soil in which 29 plants were grown.

Porto Rican red-clay soil, which is well adapted for rice, was used in the cylinders. This was fertilized liberally with sulphate of ammonia, acid phosphate, and muriate of potash, so that the ash composition might not be influenced at any stage by a lack of nutrients. Fertilizers furnishing 10 gm. of nitrogen (N), 5 gm. of phosphoric acid (P_2O_5) and 10 gm. of potash (K_2O) were incorporated with the soil before planting; when the plants were 18 days old, a surface application of 2 gm. of nitrogen, 1 gm. of phosphoric acid, and 2 gm. of potash was made; and when the plants were 59 days old, 3 gm. of nitrogen, 3 gm. of phosphoric acid, and 3 gm. of potash were applied.

[1] Kelley, W. P., and Thompson, Alice R. A study of the composition of the rice plant. Hawaii Agr. Exp. Sta. Bul. 21, 51 p. 1910.

The plants were watered only a few times, during an occasional dry spell, and made an excellent growth. The growing period was from June 15 to October 16, during which time the weather conditions were fairly uniform, with high temperature and humidity. The average monthly mean temperature ranged from 77.2° in June to 79° F. in October. The monthly precipitations from June to September were, respectively, 10.90, 11.98, 11.67, and 8.22 inches. There were, however, some dry spells of a week or 10 days that apparently affected the plants; note of this is made below.

At 18 days the plants were thinned from 40 to 29 in each cylinder, at which number they were kept during growth. The 11 plants removed from each cylinder at this time served for the 18-day-old sample, while for the 26-day-old sample 6 cylinders were cut; for the 48-day-old sample, 5 cylinders; and for the succeeding samples, 4 cylinders each. As it was impossible to remove the roots completely from the heavy clay soil, the weight of the roots is not recorded. The roots were removed, however, as completely as possible for analysis.

In preparing the samples for analysis each leaf and stalk was washed individually immediately after cutting to guard against loss of mineral matter by leaching. Under such conditions there was probably a certain loss of mineral matter from withered leaves, but no appreciable loss from the green leaves. However, this is practically of little importance, as the conditions of washing, while thorough, were no more severe than those to which the plant would be subjected by rainfall. Even digesting the leaves in cold water for 15 minutes extracted little mineral matter from green leaves. Forty-five gm. of green rice leaves previously washed on the plant were stirred up with 1 liter of distilled water. The water on evaporation yielded a residue of 0.008 gm. of mineral matter, part of which was due to minute leaf hairs broken off in the stirring; 9 gm. of withered leaves soaked for 15 minutes in 500 c. c. of water left a residue of 0.057 gm. of mineral matter.

The analytical methods employed were essentially those of the Association of Official Agricultural Chemists,[1] with a few exceptions. Preparation of the ash was by the optional method, igniting over a very low flame without calcium acetate and leaching when necessary. Iron was determined colorimetrically with potassium thiocyanate, this method being preferable to titration with potassium permanganate for the small amounts present.

ANALYTICAL RESULTS

In Table I are given the data on the weight and composition of a single plant with respect to withered leaves, etc., at each period of sampling. The weights of the plants were, of course, accurately determined, the probable error of the weights and percentages of dry matter merely show-

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

ing the degree of accuracy with which each sample represented, in respect to weight and moisture content, the average of all the plants at each period. In calculating the probable error, one cylinder of 29 plants was taken as a unit. The development of the plants at the different stages was as follows: At 18 days the plants were stooling to some extent; at 73 days they were just about to flower; at 103 days panicles were out, but the seeds were only partially formed; at 123 days seeds were fully formed and ripe.

TABLE I.—*Weights of the different parts of the upland rice plant analyzed at various periods*

Age of plant.	Dry weight of green stalks and leaves per plant.	Dry weight of withered stalks and leaves per plant.	Dry weight of panicles per plant.	Dry weight of whole plant above ground per plant.	Percentage of dry matter in whole plant aboveground.
Days.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
18	0.132	.....	.....	0.132	18.7
26	.581	.....	.....	.581±0.035	20.2±0.08
48	4.38	.....	.....	4.38 ±0.25	14.6±0.05
73	11.47	0.95	.....	12.42 ±1.20	20.9±0.10
103	21.76	4.53	3.24	29.53 ±0.99	25.8±0.99
123	.....	23.34	12.12	35.46 ±1.90	36.2±0.16

It will be noted that the percentage of dry matter in the green plant did not rise until the plant had begun to form seeds. Previous to this time the percentage of dry matter in the plant was somewhat irregular, but tended to remain about 20 per cent. The variations in the moisture content of the first four samples are so many times the probable error of each result that they could not be due to poor sampling. There is little doubt that weather conditions affected the amount of moisture or dry matter in the green plant during the first four stages of growth, while the moisture content of the last two samples was controlled chiefly by the physiological changes in the plant—accumulation of carbohydrates and death of old leaves. This seems evident from the records of rainfall. During the eight days preceding the cutting of each sample the number of days with rain and the total precipitation for the eight days were as follows: Previous to the 18-day-old sample, 5 days with rain, 2.80 inches; previous to the 26-day-old sample, 1 day with 0.90 inch; previous to the 48-day-old sample, 6 days with 4.63 inches; and previous to the 73-day-old sample, 1 day of rain with a precipitation of 0.32 inch. The weather was thus relatively wet, dry, wet, dry; and the percentages of moisture in the green plant were respectively high, low, high, low.

The ash analyses of the various samples are given in Table II. The panicles included the seeds and supporting stems. Withered leaves of the 73- and 103-day-old samples were analyzed separately from the green leaves and stalks, but no such separation was made for the 123-day-

old sample, as all the leaves and straw were partially or completely withered at this period. The 18- to 48-day-old samples had no withered leaves, so that these analyses represent the whole plant aboveground.

TABLE II.—*Ash analyses of vegetative parts of the rice plant at various periods*

Part of plant.	Age of plant.	Percentage of dry matter in green sample.	Percentages in dry matter of—		Percentages in carbon-free ash of—								
			Carbon-free ash.	Nitrogen (N).	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl_2).
Green leaves and stalks	*Days.* 18	18.7	17.75	.....	56.88	2.21	3.69	0.75	22.91	1.55	7.94	9.24	.....
Do	26	20.2	14.97	4.02	56.34	2.40	4.24	.35	17.28	9.76	6.65	5.81	4.47
Do	48	14.6	22.21	1.89	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.41	4.10
Do	73	20.1	16.95	1.93	64.82	1.73	3.04	.18	16.88	4.55	3.39	3.89	5.37
Do	103	21.5	13.28	1.01	67.71	2.12	3.26	.11	9.54	10.31	4.96	3.61	4.52
Withered leaves and stalks	123	28.7	20.23	.62	74.00	1.83	2.27	.32	12.65	4.85	1.80	2.52	3.26
Withered leaves	73	37.4	30.12	1.16	83.51	3.12	3.08	.97	6.44	1.31	1.33	1.73	1.69
Do	103	61.0	27.60	.41	85.10	3.00	2.84	.72	2.74	2.33	.67	1.20	.80
Panicles immature	103	48.5	8.86	1.18	78.15	1.17	2.68	.07	6.80	3.20	6.91	3.72	.68
Panicles with ripe seed	123	72.8	4.82	1.26	68.93	1.31	4.91	.06	9.74	1.04	14.67	5.60	.....

The percentage of iron in the ash of the green straw and leaves decreased regularly and rapidly with the maturity of the plant, the greatest decrease being from the 18-day-old to the 26-day-old sample.[1] The withered leaves had a relatively high percentage of iron. This may be due to the other samples, consisting of both leaves and stalks, or to the fact that the withered leaves of the 73- and 103-day-old sample were the leaves that appeared first—i. e., those forming a large part of the first samples.

The varying percentages of iron in the ash of the green straw and withered leaves agree with some of the results obtained by Arendt [2] with oats. He found that the lower leaves of wheat, which must have been withered at the later periods of analysis, contained increasing percentages of iron, which were much greater than the percentages of iron in the ash of the upper leaves.

The lower percentages of potash, phosphoric acid, sulphur, chlorin, and nitrogen in the ash of the withered leaves may be due to translocation of these elements preceding death of the leaves or to loss by leaching after death of the tissue.

In Table III is given the ash composition of the roots and of the whole plant aboveground. The roots for analysis were washed with great care,

[1] These results are in accord with many analyses of green rice straw made previously. Four samples of rice straw from plants grown in four different soils for 25 days contained from 2.76 to 1.98 per cent of iron (Fe_2O_3) in the ash, while samples from a crop grown 84 days had 0.31 to 0.18 per cent, and samples from a 129-day-old crop had but 0.12 to 0.10 per cent of Fe_2O_3 in the ash. (Gile, P. L., and Ageton, C. N. The effect of strongly calcareous soils on the growth and ash composition of certain plants. Porto Rico Agr. Exp. Sta. Bul. 16, p. 31, 1914.)

[2] Arendt, R. F. E., Untersuchungen über einige Vorgänge bei der Vegetation der Haferpflanze. *In* Landw. Vers. Stat., Bd. 1, p. 31–36. 1859.

but it was impossible to wash them white. The analyses show that the material which could not be washed off was probably finely divided ferric oxid. The percentages of iron found in the ash of the roots ranged from 5.36 to 8.48. This was obviously due to iron contamination from the soil. It was evident, however, that this was a selective contamination chiefly of iron particles, as the ratio of Fe_2O_3 to Al_2O_3 to SiO_2 in the soil was about 1 to 1.5 to 6.[1] Thus, a contamination of the soil as such which would have increased the iron content 6 per cent would have raised the silica 36 per cent and the alumina content 9 per cent. As the high iron content of the root ash is thought to be due to selective contamination from the soil, the results for iron are not reported. The percentages of the other constituents, except possibly silica, could not have been materially affected by soil contamination.

TABLE III.—*Ash composition of the roots and of the whole rice plant aboveground*

Material analyzed.	Age of material.	Percentages in carbon-free ash of—								
		Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl_2).
	Days.									
Whole plant aboveground.	18	56.88	2.21	3.69	0.75	22.91	1.75	7.94	9.24	.....
Do.....	26	56.34	2.40	4.24	.35	17.28	9.76	6.65	5.81	4.47
Do.....	48	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.41	4.10
Do.....	73	67.24	1.89	3.07	.28	15.54	4.13	3.10	3.61	4.90
Do.....	103	73.29	2.30	3.09	.28	7.46	7.60	3.87	2.94	3.22
Do.....	123	73.43	1.77	2.57	.29	12.33	4.43	3.21	2.86	2.90
Roots.....	18	42.28	3.82	9.68	.....	22.53	2.10	7.33	.....	.....
Do.....	26	35.62	3.75	8.42	.....	15.46	17.23	8.11	5.48	.....
Do.....	48	46.06	3.01	4.36	.....	21.03	6.32	4.98	8.06	2.30
Do.....	73	60.21	2.84	4.30	.....	15.24	3.74	3.02	6.73	1.92
Do.....	103	61.57	2.76	3.84	.....	10.83	4.42	2.46	6.67	.99
Do.....	123	64.70	4.31	3.05	.....	12.47	1.19	2.63	6.87	1.45

The percentages of iron in the ash of the whole plant aboveground showed but little variation after the sharp drop from the 18- to the 26-day-old sample.

Leaving out of consideration the 123-day-old sample, the composition of which was probably influenced appreciably by the leaching of rain, it can be seen that during the growth of the plant the percentages of lime and magnesia in the ash tended to remain constant, the silica increased, the phosphoric acid and sulphuric acid decreased, the potash, somewhat irregular, tended to decrease, and the soda was irregular. The variations in the percentages of soda are somewhat peculiar, the increase from the 18- to 26-day-old sample being out of all proportion to changes in other constituents. Soda in the ash of the roots, however, increased to an

[1] Iron is much higher in the finer soil separates than in the coarser. (Failyer, G. H., Smith, J. G., and Wade, H. R. The mineral composition of soil particles. U. S. Dept. Agr. Bur. Soils Bul. 54, 36 p. 1908.)

equally great extent from the 18- to 26-day-old sample. Variations in the percentages of potash in the ash of the plant aboveground were for the most part accompanied by similar variations in the ash of the roots. The percentages of soda in the ash seem, as a rule, to fluctuate inversely as the percentages of potash. This is in accord with results showing that soda can to a small extent replace or exercise part of the functions of potash.[1]

In the ash of the roots lime, magnesia, phosphoric acid, and chlorin all decreased fairly regularly with the age of the sample.

In Table IV are given the percentages of the ash constituents present in the dry matter of the roots and of the whole plant aboveground.

TABLE IV.—*Ash constituents in dry substance of the roots and the whole rice plant aboveground*

Material analyzed.	Age of material.	Percentage of dry matter in whole plant aboveground.	Percentages of ash constituents in dry substance of plant.										
			Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl_2).	Nitrogen (N).
Whole plant aboveground	*Days.* 18	18.7	17.75	10.10	0.39	0.65	0.133	4.07	0.28	1.41	1.64	.....	.....
Do	26	20.2	14.97	8.43	.36	.63	.052	2.59	1.47	1.00	.87	0.67	4.02
Do	48	14.6	22.21	13.89	.38	.69	.048	3.26	2.13	.89	.98	.91	1.89
Do	73	20.9	17.96	12.07	.34	.55	.051	2.79	.74	.56	.65	.88	1.87
Do	103	25.8	14.99	10.99	.35	.46	.041	1.12	1.14	.58	.44	.48	.94
Do	123	36.2	14.96	10.99	.26	.38	.044	1.85	.66	.48	.43	.43	.84
Roots	18	.....	11.71	4.95	.45	1.13	.....	2.64	.25	.86	.....	.....	.....
Do	26	.....	9.49	3.38	.36	.80	.....	1.47	1.64	.77	.52	.....	1.48
Do	48	.....	7.82	3.60	.24	.34	.....	1.64	.49	.39	.63	.18	.95
Do	73	.....	8.32	5.01	.24	.36	.....	1.27	.31	.25	.56	.16	1.09
Do	103	.....	8.09	4.98	.22	.31	.....	.88	.36	.20	.54	.08	.75
Do	123	.....	5.53	3.58	.24	.17	.....	.69	.07	.15	.38	.08	.66

In the first four samples the percentages of ash in the dry matter of the plant aboveground varied inversely as the percentages of dry matter in the green plant, and, as noted above, the percentages of dry matter seemed to be lower during the periods of greater precipitation. Thus, with dry weather preceding the sample, the percentage of dry matter in the green plant was high and the percentage of ash low.[2] An average of several crops of rice grown at different times to eliminate the effect of temporary weather conditions would doubtless show gradually increasing percentages of dry matter in the green plant and gradually decreasing percentages of total ash in the dry matter.

[1] Wagner, Paul. Forschungen auf dem Gebiete der Pflanzenernährung. I. Theil: Die Stickstoffdüngung der Landwirthschaftlichen Kulturpflanzen. p. 231, Berlin, 1892.

Hartwell, B. L., and Pember, F. R. Sodium as a partial substitute for potassium. *In* R. I. Agr. Exp. Sta. 21st Ann. Rpt., 1907–1908, p. 243–247. 1908.

[2] This is probably owing to the fact that during wet weather the growth of new leaves and tissues is especially active, while in dry weather organic matter is formed more rapidly than mineral matter is absorbed.

On account of the fluctuations in the amount of total ash, it is thought that the percentages of the various ash constituents in the dry matter are less significant than the composition of the ash, which would be unaffected by temporary weather conditions.

The plants were not analyzed at frequent intervals while ripening; nevertheless, the preceding work throws some light on the question of loss of mineral elements at this time. In Table V are given the absolute weights of the ash constituents in one plant at 103 and at 123 days.

TABLE V.—*Gain or loss of ash constituents by the rice plant aboveground during last 20 days of growth*

Material analyzed.	Age of material.	Weight of ash constituents (in grams) in one whole plant aboveground.										
		Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl_2).	Nitrogen (N).
	Days.											
Whole plant aboveground..	103	4.427	3.245	0.102	0.137	0.012	0.330	0.337	0.172	0.277	0.130	0.143
Do.....	123	5.306	3.896	.094	.137	.015	.655	.235	.170	.297	.152	.154

It is evident that the aboveground part of the plant lost considerable soda between the last two periods. The roots also must have lost considerable soda, as the percentage of soda in the dry matter of the roots dropped from 0.36 per cent at 103 days to 0.07 per cent at 123 days, while the absolute weight of roots could have increased but little during this interval. The results do not show whether there was any loss of the remaining ash constituents. It is only apparent that, as compared with 103 days, the plant aboveground contained at 123 days the same or a slightly greater quantity of all ash constituents except soda. It is, of course, possible that between 103 and 123 days there might have been an increase followed by a loss of the other ash constituents. The marked loss of soda was more than compensated for by a gain in potash. The increases in the other elements were relatively slight, and the apparent losses of lime and phosphoric acid are without significance when the probable errors of the weights of the plant at the two periods are considered.

DISCUSSION OF RESULTS

It is unnecessary to detail all the changes in ash composition that occurred during the growth of the plant, as these are evident in the tables. In common with similar studies of many other plants the percentages of potash, phosphoric acid, and sulphur in the ash and of nitrogen in the dry matter decreased with the age of the plant, while the silica increased.

The results show that while the iron content of the ash of the whole plant varied but little with the age of the plant, the percentage of iron in

the ash of the green straw and leaves decreased markedly with its age. The withered leaves and straw thus contain a much greater percentage of iron in the ash than the active or live parts of the plants. This would indicate that iron, like silica, is not transported or leached from the dead tissue to the same extent as the other mineral elements.

SUMMARY

Ash analyses of upland rice were made at intervals to show the ash composition of the plant, especially in regard to iron content, from an early stage to complete maturity.

The percentages of potash, phosphoric acid, and sulphur in the ash of the whole plant aboveground decreased with the age of the plant, while silica increased and nitrogen in the dry matter decreased with the age.

As compared with 103 days, when the panicles were just out, the mature plant aboveground at 123 days with the seeds ripe contained an equal amount of lime, magnesia, and phosphoric acid, slightly more iron, sulphur, chlorin, nitrogen, and silica, much less soda, and considerably more potash.

The percentages of iron in the ash of the green leaves and straw decreased regularly and markedly with the age of the plant, while the percentages of iron in the ash of the whole plant aboveground remained fairly constant after the 26-day-old sample.

Previous to flowering, the percentages of dry matter in the green plant and of ash in the dry matter seemed to be influenced by the effect of the weather on the growth of the plant.

VARIETAL RESISTANCE OF PLUMS TO BROWN-ROT

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INTRODUCTION

In the control of plant parasites a great deal of attention has recently been paid to the possibilities of producing resistant plants by breeding. In the plum-breeding plots of the Minnesota Fruit-Breeding Station at Excelsior it is very noticeable that the fruit of certain seedling varieties of plums (*Prunus* spp.) appears to rot much more readily than that of others. The rot is due to attacks of the brown-rot fungus, *Sclerotinia cinerea* (Bon.) Wor. As a knowledge of the factors controlling resistance is necessary for intelligent effort in breeding work, a study of the resistance of plums to the brown-rot fungus was begun in the spring of 1913. The following is a report of the results obtained on the nature of parasitism of the fungus and on varietal resistance of plums to the fungus.

HISTORICAL SUMMARY

TAXONOMIC REVIEW

The life history of the brown-rot fungus has been rather completely worked out, both in this country and in Europe. Woronin (1900)[2] made a very complete comparative study of *Monilia fructigena* and *M. cinerea*. Two years later Norton (1902) discovered and described the apothecial stage of the American form and referred *M. fructigena* Persoon to *S. fructigena* (Pers.) Schröter. Shortly after this, Aderhold and Ruhland (1905) found and described a perfect stage of *Sclerotinia* spp. on apples, which they concluded to be that of *M. fructigena*. They also found a perfect stage of the apricot brown-rot fungus, *M. laxa*, the Monilia stage of which can not be distinguished morphologically from that of *M. cinerea*. A comparison of the perfect stage of the apricot fungus with the perfect stage of the peach fungus of this country, sent to them by Norton, showed differences in ascus and ascospore sizes, and these, with the slight differences which they found in the ability of the two species, *S. cinerea* and *S. laxa*, to infect plum flowers, led them to the conclusion

[1] The work was carried on under direction of the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota. The writer wishes to acknowledge indebtedness for suggestions, assistance, and criticism to the following: Dr. E. M. Freeman and Dr. E. C. Stakman, Prof. R. W. Thatcher, of the Division of Chemistry, and Dr. M. J. Dorsey, of the Division of Horticulture, in whose laboratory the work was carried on. The writer also wishes to express his appreciation of the assistance rendered by Mr. Ernest Dorsey in the photomicrographic work and to Dr. C. O. Rosendahl for suggestions and the use of apparatus.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 392–395.

that the fungus found on the apricot was a species (*S. laxa*) distinct from that found on plums and cherries (*S. cinerea*). They also concluded that the American species must be *S. cinerea*. A comparison of the ascospores of *S. cinerea* with those of *S. fructigena* brought out the fact that the former always contain from one to many oil globules, while the latter contain none.

Pollock (1909), in a study of the Michigan brown-rot fungus, concluded that it was probably the same species which Norton described, and that, so far as the chlamydospore measurements were concerned, it resembled *S. cinerea* more than *S. fructigena*. Pollock also showed that the microconidia observed by Woronin (1888) on certain other species of Sclerotinia and by Humphrey (1891) as appearing on plums which did not produce spore tufts were also produced in abundance when ascospores of the American brown-rot fungus were germinated in distilled water.[1]

An important taxonomic fact was brought out by Ewert (1912) when he showed that the Monilia spores of *S. fructigena* would not live over the winter, while those of *S. cinerea* would. This difference was not due to the effects of cold, as the spores of *S. fructigena* would stand low temperatures. That the spores of the American form would live over the winter was shown by Arthur (1886), who on May 8 germinated spores taken from mummies of cherries which had hung on the tree all winter. Galloway (1889), in May, 1888, germinated spores taken from mummies collected in July, 1886.[2]

The perfect stage of the cherry brown-rot fungus in Europe was not found until 1912. Westerdijk (1912) described it at this time and concluded (p. 41), from ascus and ascospore measurements, that "Neben den 3 beschriebenen Obstsclerotinien ist dann also eine spezielle Kirschensclerotinie aufzustellen." The asci and ascospore measurements presented by Reade (1908) and Pollock (1909), however, do not warrant this conclusion.

Matheny (1913) made an extensive study of the brown-rot fungus from various parts of this country and compared it closely with pure cultures of *S. fructigena* and *S. cinerea* sent to him from Europe. He concluded that the Monilia stage in this country agreed very closely with that of *S. cinerea* of Europe and that the apothecial stage differed in shape of spore and in the presence of oil globules in the ascospores from that of *S. fructigena* and referred the American brown-rot fungus to *S. cinerea*. Conel (1914) made a study of the brown-rot in the vicinity of Champaign and Urbana, Ill., and decided, both because of its morphological characters and from the fact that the Monilia form is capable of living over winter, that the fungus was *S. cinerea*.

[1] Jehle in an unpublished thesis on file at the University of Minnesota also observed the production of these conidia from ascospores, and on the same hypha observed the Monilia spores, thereby definitely connecting the perfect and the Monilia stages.

[2] Jehle also germinated spores found on mummies in the early spring.

PHYSIOLOGICAL REVIEW

A considerable amount of literature has appeared, especially in recent years, on the subject of resistance and immunity to disease. The cereal crops have perhaps received the most attention. Bolley (1889) and Anderson (1890) attempted to correlate resistance with certain morphological characters. Cobb (1892, p. 181–212) advanced the theory of mechanical resistance due to morphological characters, such as thick cuticle, waxy coating, and small stomata. Freeman (1911) showed that barley might escape rust owing to variation in amount of bloom produced on the leaves, which could be varied by growing in soils of different degrees of alkalinity. This escape from rust is not true resistance, but is due to the inability of the water to wet the surface of the leaves so that the drops containing the spores roll off. When these plants were infected, however, they "exhibited large and vigorous growths of the rust."

Marryat (1907) showed in the case of *Puccinia glumarum* grown on a semi-immune host that it killed small areas of the host tissue and formed only small or abortive pustules, while in the case of the susceptible forms the host cells, though containing haustoria, were apparently normal.

Comes (1912) reported that Rieti wheat, which is very resistant to rust, contained a higher percentage of acid than other more susceptible forms and also that the acid content increases with the altitude at which wheats are grown, as does also the ability to resist rust.

Jones (1905) showed that some varieties of potatoes are much more resistant to certain potato diseases than others. He based resistance more on chemical composition than on morphological differences in the host.

Kinney (1897) noted that "fruit of different varieties of plums varies in susceptibility to injury by rot fungus" and attributed the difference in resistance to variations in texture of the skin. He also stated that early varieties are usually injured more than those which ripen their fruit later.

Müller-Thurgau (1900) noticed that varieties of apples in Switzerland showed different degrees of susceptibility to a wilt or blight caused by *M. fructigena*.

Quaintance (1900) observed a marked variation among varieties of drupaceous fruits in their resistance to attacks of the brown-rot fungus. Among the peaches the varieties densely covered with down were the most susceptible. Of the plums some varieties of the Miner group were practically free, those of the Wild Goose rotted about 10 per cent, while the varieties of *Prunus americana*, *P. triflora*, and *P. pumila* were very susceptible. He suggested that the firmness and thickness of the skin of the Miner plums might have something to do with their resistance. The relative resistance of some varieties of *P. domestica* to brown-rot is given by Alwood and Price (1903).

Köck (1910) ascribes the resistance of certain varieties of cherries to a blossom-blight caused by *S. cinerea* to the blossoming of these varieties when conditions are unfavorable for the disease.

Cook and Taubenhaus (1911 and 1912) pointed out the toxic properties of tannins and fruit acids and also showed a relationship to exist between the decrease in oxidizing enzym content of fruits and the increase in their susceptibility to disease.

With regard to the physiological relationship between host and parasite, considerable work has been done. Jones (1910) gave a comprehensive review of the literature on this subject, dealing especially with the bacteria. Cooley (1914) reviewed much of the work on the physiological relations of the fungi. Therefore, only a short review will be given of the literature dealing with *Sclerotinia* spp.

Behrens (1898) in his work on the physiology of *Oidium* (*Sclerotinia*) *fructigenum*, *Penicillium* spp., and some other fungi, concluded that *S. fructigenum* was exclusively an intercellular fungus and did not secrete a cellulose-dissolving enzym. He considered that the fungus split the middle lamella by mechanical force. *Penicillium* spp., he concluded, also did not enter the cells, but did produce a middle-lamella-splitting enzym.

Schellenberg (1908) studied the effect of *S. fructigena* and *S. cinerea* on a number of tissues, but not on their respective hosts. He considered both of these fungi to be intercellular, producing no cellulose-splitting enzym. He thought, however, that they did produce a hemicellulose-dissolving enzym and that the cell walls in contact with the hyphæ were slightly dissolved. He saw no evidences of a middle-lamella-splitting enzym.

Bruschi (1912) noticed, when *M. cinerea* was grown in a medium containing plum flesh, that after 48 hours the cells were all separated from one another, and concluded that the fungus produced the middle-lamella-splitting enzym pectinase. Attempts to isolate a cellulose-dissolving enzym were unsuccessful.

Cooley (1914) demonstrated the ability of *S. cinerea* to produce an enzym which would coagulate pectin from solution in the absence of calcium. This enzym he called "pectinase." His use of this term is, however, not clear, as he states (p. 314) that he adopted "the nomenclature used by Jones and Euler, namely, employing pectinase as the term to designate the enzyme inducing coagulation of a pectin solution and also the hydrolysis of calcium pectate, or pectinate." Jones (1910) used, in a general way, the nomenclature suggested by Bourquelot and Hérissey (1898) regarding the enzym which they extracted from barley malt; as he says (p. 355), "All things considered, we favor the name *pectinase*, which was suggested by Bourquelot and Hérissey, as already explained." On the other hand, Euler-Chelpin (1912, p. 32) states that "The enzyme

here termed pectase was obtained from malt-extract by Bourquelot and Hérissey, who called it pectinase; according to the general principle of naming enzymes after the substrate, this should be altered to pectase." In a subsequent paragraph he states that "By the term pectinase should be indicated the enzyme which coagulates dissolved pectin substances, e. g., in fruit juices, in the presence of lime to gelatinous calcium salts of the feebly acid pectinic acids." If we follow the definition of a pectinase given by Jones and the classification given by Haas and Hill (1913, p. 339), we must refer to the enzym demonstrated by Cooley as "pectase."

The attempts of Cooley (1914) to isolate a middle-lamella-splitting enzym from rotted fruit gave negative results. In certain artificial media a cellulose-dissolving enzym was produced, but its action on cellulose isolated from plums was very slight. From direct observations on the fungus in free-hand sections of fruit he concluded that "the fungus does not show any particular affinity for the middle-lamella, but penetrates and permeates with equal avidity any part of the host tissue." He could find no relationship to exist between varying acid content of plums at different periods of development and increased susceptibility of ripe over green fruits.

EXPERIMENTAL MATERIAL

The organism used in this work was isolated when needed from rotting plums, as it seemed better to use only strains which had been growing under normal conditions rather than to risk a decrease in virulence of infection due to growing a single strain on artificial media.

The plums used consisted for the most part of hybrids produced at the Minnesota Fruit-Breeding Station at Excelsior. Those referred to in the text as "B × W" are hybrids of Burbank (*P. triflora*), the female parent, with Wolf (*P. americana mollis*). The A × W crosses are Abundance (*P. triflora*) × Wolf. The Burbank is a medium thick-skinned variety which becomes soft when ripe and is rather susceptible to the brown-rot. Wolf has a thick, tough skin and is not affected to any great extent by the rot in the field. Abundance is reported by Hedrick (1911) as being less subject to attacks of the brown-rot than Burbank. The crosses B × W15 and A × W18 are both characterized by being very firm when ripe, and are both nearly immune to brown-rot in the field. The other hybrids of these two series vary in firmness and resistance.

Etopa and Sapa (*Prunus besseyi* × Sultan, *P. triflora*) and Wakapa (Red June, *P. triflora*, × DeSota, *P. americana*, but resembling very closely a sand-cherry hybrid) are products of the South Dakota Experiment Station. They are thin-skinned varieties and are susceptible to rot. The sand cherry (*P. besseyi*) is a small fruit which becomes soft on ripening. It has very astringent flesh and is susceptible to brown-rot.

Gold is a thin-skinned susceptible variety. Sultan is not known to the writer.

The three varieties designated "S. D. Nos. 1, 2, and 3" are varieties obtained from Mr. A. Brackett, of Excelsior, who received them from the South Dakota Experiment Station. Their true names were not known to Mr. Brackett. S. D. No. 1 is a thin-skinned variety and rotted badly on the trees when sprayed once with Bordeaux mixture. S. D. Nos. 2 and 3 were thicker skinned, firmer varieties and did not rot after one spraying, many fruits drying on the trees. All appear to be sand-cherry hybrids.

Compass, a hybrid between a sand cherry and *P. americana* (Hansen, 1911), is a thin-skinned variety which becomes soft on ripening and is susceptible to the brown-rot. Reagan, a hybrid of Wayland (*P. hortulana*) × *P. americana* (Hedrick, 1911) is thick-skinned, very firm when ripe, and is very resistant to the rot. Specimens of the ripe fruit used were received from the New York Experiment Station, Geneva, N. Y.

Ocheeda and Harrison are varieties of *P. americana*. Manitoba No. 1 is probably a variety of *P. nigra*. Hammer is a hybrid between *P. hortulana mineri* and *P. americana* (Hedrick, 1911). These varieties were obtained from the orchard at University Farm.

TAXONOMY OF THE FUNGUS

MONILIA STAGE

The brown-rot fungus in Minnesota is found for the most part affecting plums, but to a very limited extent also attacking the apple. It appears on the plum first as a small brown or purple spot, which increases very rapidly in size. In a very short time the spore tufts appear irregularly over the surface of the rotted area. These are usually small and ashen gray in color, although in many cases the color varies to a yellow ocher. Plums inoculated through a wound made by cutting off the tip of the fruit, when allowed to rot under a cardboard box in nearly total darkness, produced spores of a bright-ocher color over the wounded area and in some cases through the skin. Mummies collected from trees in the late fall showed spore tufts which varied from gray to a light ocher. The chlamydospores of the local form, taken from mummies which have hung on the trees over winter, retain their power of germination.

Chlamydospore measurements were made of spores from Soulard and Longfield apples, from Harrison, Ocheeda, Newman, and Surprise plums, which were rotted in the laboratory, and from a culture on beerwort agar. In each instance 100 spores were measured, except in the case of the beerwort-agar culture, where 50 spores were measured. The results are given in Table I.

TABLE I.—*Chlamydospore measurements of Sclerotinia cinerea*

Medium.	Average length.	Average breadth.	Medium.	Average length.	Average breadth.
	μ	μ		μ	μ
Surprise plum	16.22	11.24	Longfield apple	15.80	10.81
Newman plum	17.38	12.10	Soulard apple	15.30	10.76
Ocheeda plum	16.18	11.09	Beerwort agar	14.05	8.77
Harrison plum	15.95	10.98			

From a comparison of these measurements with those given in Table II, it will be seen that they agree very closely with those obtained by other investigators in this country and are only slightly larger than those given for *S. cinerea* by European investigators. They also correspond closely to the measurements given by Aderhold and Ruhland for *S. laxa* found on apricots.

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators*

FROM EUROPEAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
Sclerotinia cinerea:		μ	μ	μ
Saccardo (1886)	.....	15 to 17 by 10 to 12	.....	.....
Woronin (1900)	.....	12.1 by 8.8 to 13.2 by 9.9	.....	.....
	In culture	17.5 to 24.2 by 11.2 to 13.2	.....	.....
Aderhold and Ruhland (1905)	Cherry	13.8 by 9.2	.....	.....
	Various	13.8 by 9.95	.....	.....
Matheny (1913)	Peach and plum	14.4 by 10.8	.....	.....
Sclerotinia laxa:				
Aderhold and Ruhland (1905)	Apricot	16.1 by 10.8	121.5 to 149.9 by 8.5 to 11.8	11.5 to 13.5 by 5.2 to 6.9
Cherry brown-rot:				
Westerdijk (1912)	Cherry	.....	158.4 to 171.6 by 7.9 to 8.5	13.2 to 16.8 by 4.3 to 5.2
Sclerotinia fructigena:				
Saccardo (1886)	.....	25 by 10 to 12	.....	.....
Woronin (1900)	Apple	20.9 by 12.4 to 24.5 by 13.2	.....	.....
	In culture	23.7 to 30.8 by 14.9 to 16.5	.....	.....
Aderhold and Ruhland (1905)	Apple	25 by 13	120 to 180 by 9 to 12	11 to 12.5 by 5.6 to 6.8
Matheny (1913)	.....	22.1 by 11.2	.....	.....

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators*—Continued

FROM AMERICAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
		μ	μ	μ
Sclerotinia fructigena: Norton (1902)			45 to 60 by 3 to 4	
Aderhold and Ruhland (1905)			89.3 to 107.6 by 5.9 to 6.8	6.2 to 9.3 by 3.1 to 4.6
Reade (1908)		17 by 11	125 to 215 by 7 to 10	10 to 15 by 5 to 8
Pollock (1909)	Plum	14.4 to 24 by 9.6 to 14.4	130 to 179 by 9.2 to 11.5	11.4 to 14.4 by 5 to 7
	In culture	9.6 to 14.4 by 7.2 to 10.8		
Matheny (1913)		14.7 by 9.9		
	Peach		135 to 190 by 6.9 to 10.5	10.5 to 14.5 by 5.2 to 7.5
	Plum		135 to 173 by 6.8 to 10.8	9.3 to 14.2 by 5 to 7.4

SCLEROTINIA STAGE

The apothecial stage of the local brown-rot fungus has been found in abundance in the University of Minnesota Experiment Station orchard during the last few springs. It appears during the blooming period of the plums. The ascospores showed the characteristic refractive globules which Aderhold and Ruhland (1905) pointed out as being one of the characters which make it possible to distinguish between *S. cinerea* and *S. fructigena*, the latter species containing none.

Some doubt has existed in regard to the exact time required for the production of the perfect stage after the formation of the sclerotium or mummy. The field observations of Norton (1902) and others seem to indicate that the apothecia are formed the second spring after the rotting of the fruit—i. e., in approximately 18 months. Other investigators (Dandeno, 1908) have thought that they may be produced the spring following the rotting of the fruit. No experimental evidence has come to the notice of the writer which shows definitely the period required for the production of apothecia; therefore, the following experiment was performed.

During the fall of 1913 two lots of mummied plums and one of apples were buried. Lot 1 consisted of 1 plum each of 16 varieties which had been rotted in the laboratory. These were buried on October 8, 1913, about ½ to 1 inch deep in a shallow box, which was then placed level with the ground on a shaded hillside. Lot 2 consisted of (A) 106 fruits from 8 varieties of plums which had rotted in the field under field conditions during the fall of 1913, and (B) 30 mummies of 3 other varieties which

had been hanging on the trees since the fall of 1912. The plums of this lot were buried on October 15, 1913, near the previous lot and when finally examined were buried from ¼ to 1 inch deep. The fruit of each variety was kept separate. Lot 3 was made up of 48 apples representing 7 varieties. The fruits had been inoculated through wounds in the laboratory and on October 18, 1913, when entirely rotted, were buried.

The results obtained were as follows: In the spring of 1914 no apothecia were found on any of the three lots. An examination of lot 1 on May 7, 1915, showed 4 of the total of 16 fruits producing a total of 71 cups. On further examination these were all found to be growing from the upper side of the sclerotium. Two others, which had been buried deeper, were found to be producing many of the young cups which at this time had not reached the surface of the ground.

Lot 3 at this time showed no apothecia. On May 12, 1915, lot 2 was examined; of the total of 106 mummies produced in 1913, 39 were producing apothecia in abundance. In a number of other instances the sclerotium was present, but was producing no apothecia. Of the 30 mummies produced in 1912, 4, of the Opata variety, were producing a total of 10 cups, while the sclerotia of the Compass and Topa varieties had entirely rotted. At this time lot 3 was also examined, and as no apothecia were being produced an attempt was made to find the sclerotia. Small pieces of the black, leather-like sclerotia were found where 4 of the varieties had been buried, but in all other cases they had entirely rotted. The sclerotium of a Shields crab-apple had a growth of about one-fourth of an inch upon it which appeared very much like that of a young cup, but when this piece was again buried it showed no further development.

From this experiment we may conclude that for the production of the perfect stage of *S. cinerea* the mummies must be buried for at least two winters and that mummies which have hung on the tree for one year still have the power of producing apothecia.

From a horticultural standpoint it is of interest to note that of the 156 plum pits buried in 1913 none germinated in the spring of 1914, but in the following spring 106 produced young plants. Of these, 6 were of the Topa variety which had hung on the tree for one year before burying.

Measurements were made of asci and ascospores from material collected on April 10, 1914. The asci varied in length from 102 to 166μ, and in breadth from 3.5 to 5.7μ. The ascospores varied from 5.6 to 8.9μ in length and from 2.9 to 3.8μ in breadth.

Reference to Table II shows the wide range in ascus and ascospore measurements as determined by various investigators, the asci of Norton ranging from 45 to 60 by 3 to 4μ; of Aderhold and Ruhland (who received their material from Norton), 89.3 to 107.6 by 5.9 to 6.8μ, those from the Minnesota Experiment Station, 102 to 166 by 3.5 to 5.7μ, while the upper extreme is reached by Reade (who also obtained his material from Norton), who found the asci ranging from 125 to 215 by 7 to 10μ.

By comparing the figures given by Westerdijk (1912) for the cherry fungus with those given above, it will be seen that they fall well within the range of *S. cinerea*, and as this difference in the size of the asci and of the ascospores was the only one upon which she based her conclusion as to its being a separate form, it seems safe to conclude that what she described was the perfect stage of *S. cinerea*.

It has already been pointed out that the Monilia stage of the apricot fungus, described by Aderhold and Ruhland (1905), compares favorably with the Monilia stage of the American brown-rot fungus, and they showed that it was identical, except for slight differences in chlamydospore size, with that of the European *S. cinerea*. By referring to Table II it will be seen that the ascus and ascospore measurements given for the perfect stage of *S. laxa* fall well within the limits determined for *S. cinerea*. Considering the fact that at present there are no known morphological differences between *S. cinerea* and the apricot fungus, is the fact that Aderhold and Ruhland were able to get infection of plum flowers in only a few cases with chlamydospores of *S. laxa* sufficient evidence to make this a separate species?

MICROCONIDIAL STAGE

The microconidial stage, as was stated above, has been described by Woronin for a number of species of Sclerotinia, including *S. fructigena* and *S. cinerea*. He, however, could show no differences between the spores of the two latter species, and they are therefore of little value in identification of the species.

The production of the microcondia was first seen by the writer in a potato-plug culture of the local fungus nearly a year old. The spores ranged from 2.2 to 2.6μ in diameter, were spherical, and contained a large refractive globule. They were later found on agar cultures in great abundance, in hanging drops of distilled water, and also in hanging drops of 1 per cent malic, 0.062 gallic, 0.062 and 0.25 per cent tannic acids. In the latter cases the flask-shaped sterigmata could be seen. Chains of from 15 to 20 spores were not uncommon. They were also produced in great abundance on the surface of a very young Surprise plum picked and inoculated June 3. These spores ranged in size from 2.55μ to 3.22μ, averaging for 25 measurements 2.72μ. The microconidia produced in the 1 per cent malic-acid solution were larger, ranging from 2.60 to 3.79μ, measurements of 25 spores averaging 3.14μ.

PHYSIOLOGICAL AND PATHOLOGICAL RELATIONS

INFECTION

Opinions differ as to the ability of the brown-rot fungus to penetrate the uninjured surface of fruits. Peck (1881) was unable to get infection of fruits when the spores were planted on the uninjured surface. Smith

(1889), however, had no trouble in bringing about infection in ripe peaches when he sowed the spores in a drop of water on the uninjured skin. Cordley (1899) obtained similar results with plums and cherries.

Field observations indicate that infection of green plums may take place through the uninjured surface if conditions are very favorable. These cases are comparatively rare, the greatest number of infections in green fruit taking place through curculio or other wounds. It is not rare, however, to find in a rotting condition uninjured green plums which are in contact with a rotting plum that is producing spores. In the ripe fruit it is not at all uncommon to find rot due to infection through uninjured cuticle which is not in contact with that of other plums.

Cooley (1914, p. 322–323) concluded from infection experiments that "The brown-rot organism will infect fruits which are immature, even penetrating those which are not more than half-grown or those in which the pits are still soft, provided the skin is punctured." He had no trouble in infecting ripe fruits without injuring them.

In the following infection experiments, carried on to determine the relative resistance of varieties, results were obtained which differ somewhat from those of Cooley.

On June 14, 1913, five plums of each of seven varieties were put into a sterile chamber and sprayed with distilled water containing Monilia spores. The results are set forth in Table III.

TABLE III.—*Results of inoculation of green plums with Sclerotinia cinerea through uninjured cuticle*

Variety.	June 14.	June 16.	June 17.
Etopa.....	Plums inoculated...	1 infection spot.....	5 infection spots on 2 plums.
Opata.....	.....do.....	.....	5 fruits rotting.
Topa.....	.....do.....	10 infection spots...	3 fruits completely rotted; 2 have 1 spot each.
A × W 15...	.....do.....	15 infection spots...	Spots spreading slowly.
B × W 21...	.....do.....	No infection spots...	2 clean; 3 one spot each; not spreading rapidly.
B × W 15...	.....do.....	.....do.....	No infection spots.
Americana seedling No. 1.	..... do.....	1 through curculio wound.	4 clean; 1 completely rotted through curculio wound.

These results show very clearly that infection can take place through the injured skin of very young plums. This experiment was repeated from time to time until the plums were ripe, and at no time, if the temperature was favorable, was any difficulty encountered in obtaining infection through the uninjured surface of certain varieties.

The results given in Table III also indicate that there is considerable difference in the ease with which the varieties of plums are infected, as well as the rapidity with which the fruit rots after infection has taken

place. Is the difference in susceptibility to infection due to differences in morphological characters of the epidermis?

It has been definitely proved from time to time that the fungus has the ability to "bore" through the uninjured skin of plums and peaches. Therefore, penetration must take place either through the rather thick cuticle of the epidermal layer or through the stomata.

Morphology of the Skin and Flesh of the Plum

For a better understanding of the entrance and penetration of the fungus in the plum fruit, a knowledge of the morphology of the "skin" and underlying layers of cells is necessary.

Stomata.—The epidermis of the plum consists of a single layer of cells covered by a rather thick layer of a cutinized substance (Pl. XXXVIII, fig. 2). On the surface of this is secreted a waxy "bloom."

Stomata are present in the young fruit. In fruit about half grown changes take place in the stomata leading to the formation of lenticels.

The lenticels are formed in at least three ways:

(A) In some cases a few flat disk-shaped cells are formed parallel to the epidermis and lining the stomatal cavity. The walls of these cells appear to be of the same material as those of the deeper lying parenchyma cells (Pl. XXXVII, fig. 1). The guard cells often open wide and dry out. In other cases changes take place in the composition of the walls of about two layers of cells lining the stomatal cavity. These cells, the walls of which were originally cellulose, give the characteristic yellow staining reaction of cork with the iron-alum-hematoxylin safranin stain (Pl. XXXVII, fig. 3).

(B) In some varieties meristematic tissue develops from the parenchyma cells and produces tissue which partially (Pl. XXXVII, fig. 2) or completely fills the stomatal cavity (Pl. XXXVII, fig. 4). Occasionally a column of cells even grows out through the stomatal opening. These cells appear to be of the same nature as the hypodermal cells underlying the epidermis, in no case giving the staining reaction of cork.

(C) The lenticels, which appear as large, corky specks on the surface of ripe plums, are made of a pad of corky cells lying parallel to the epidermis. They probably develop at the stomata, splitting the guard cells apart and growing out through the opening. The details of their formation, however, have not been carefully studied in this connection, as only very few were encountered in the material examined.

Hypodermal Parenchyma.—Directly underlying the epidermis are layers of oblong cells slightly larger than and lying parallel to the epidermal layer. These make up what is commonly known as the "skin" of the plum. In some of the thick-skinned varieties there are often as many as seven or eight layers of these cells (Pl. XXXVIII, fig. 5), while in the thin-skinned forms often not more than one or two layers are present (Pl. XXXVII, fig. 1, 2, and 5).

Lying below the hypodermal layers of cells and in sharp contrast to them are the large, isodiametric cells which make up the mass of the fruit tissue (Pl. XXXVII, fig. 6). In the ripening process in those varieties which become soft these cells split apart at the middle lamella (Pl. XXXVII, fig. 5). The solution of the middle lamella apparently takes place more readily in these cells than in those of the hypodermal layers.

METHOD OF ENTRANCE OF THE FUNGUS

Two methods were used in the determination of the details of the entrance of the fungus. The first consisted of macroscopic observations on ripe or nearly ripe fruit shortly after infection had taken place. In the second method fruits of a number of varieties of plums at various stages of development were brought into the laboratory and inoculated, in some cases by a suspension of spores in water and in others by laying the plums in contact with moist mummies well covered with spores. After infection had taken place and small decayed spots had appeared, blocks of the flesh, including these spots, were killed and embedded in paraffin, according to the usual methods employed. These were later sectioned, mounted, and stained. Sections 8 to 11μ thick were found most satisfactory. Various stains were used, including Harper's short modification of the triple stain, Heidenhain's iron-alum-hematoxylin, and also a modification of this in which safranin was used. This last-named stain proved very satisfactory.

It was noticed continually, particularly in ripe or nearly ripe fruit, that when infection took place through the uninjured skin, the spot always had in its center a lenticel or "dot." These observations indicated that infection takes place, not through the cuticle, but through the lenticel in ripe or nearly ripe fruit. Further evidence was obtained on this point when sections were made of the skin from material in which the lenticels were either forming or completely formed and through which infection had taken place. It was found that the hyphæ entered between the guard cells into the stomatal cavity (Pl. XXXVIII, fig. 3, 4, and 5). In those stomata lined with corky material infection of the fruit tissue does not take place immediately, as the fungus apparently has not the power to pierce directly through the corky cells. The hyphæ continue to grow, filling up the stomatal cavity, and eventually exert enough pressure to split away the epidermis from the lenticel cells (Pl. XXXVIII, fig. 5). It is through this opening that infection takes place into the fruit tissue (Pl. XXXVIII, fig. 1 and 2).

In the young plums, before corky material has been formed, the germ tubes also enter through the stomata. After entering they come in contact with normal fruit tissue, and direct infection takes place (Pl. XXXVIII, fig. 4). In all, 44 instances of infection through stomata or lenticels were noted, and although the surface of both ripe and green plums was often

well covered with germinating spores, no instances were found in which the germ tubes gained entrance directly through the cuticle.

Further evidence that the germ tubes do not usually penetrate the cuticle was obtained when two green plums of B×W 15, a very resistant variety, were scraped lightly with a sharp knife, thereby removing the cuticle without otherwise injuring the epidermis, and were then inoculated. These, with seven others of the same variety which had not been so treated, were sprayed with distilled water containing chlamydospores and put under a bell jar. At the end of 58 hours the two plums which had been scraped showed 10 and 13 spots, respectively, but rotted very slowly from the infection points. The seven unscraped plums were at this time without infection spots, but eventually three of these showed evidences of infection.

Because of this method of infection, resistance can not be attributed entirely to morphological differences in the epidermis of the varieties. There are however, certain morphological differences in the stomata and lenticels which contribute to resistance, the nature of which will be discussed later. When once the fungus has gained entrance the plums always rot more or less rapidly, depending upon the variety.

FIELD OBSERVATIONS

It is apparent from the facts given that the small amount of rot found in the orchard on green plums is not due to any greater resistance to infection which the green fruit may possess over ripe fruit. Nevertheless, the brown-rot in the orchard causes the greatest damage as a ripe-rot rather than as a green-rot.

It is a fact of considerable importance that it is not until the plums are ripe and begin to soften slightly that the fungus does its greatest damage as a ripe-rot. This is due probably to two reasons. The first is that there are greater possibilities of infection at this time. Field observations show that green plums will rot on the trees, owing usually to infection through curculio or other wounds, and that the rot will spread from one to another where they are in contact. Thus the number of rotted fruits and hence of infection sources to the ripe fruit is gradually increasing. Although there are other methods of infection, the largest number in ripe fruit is due directly or indirectly to contact with rotten green plums. It is very common in the field to find large groups of plums on a tree completely rotted, while other groups on the same tree are entirely free from rot. In these groups it is nearly always possible to trace the original source of infection back to one plum which has in most cases been infected through a wound of some kind while still green.

Another source of infection, more common in ripe or nearly ripe fruits than in green fruits, is direct infection from spore suspensions in water,

due probably to the greater number of spores being produced. This is not of considerable importance, however, except under extremely favorable weather conditions, when it may be the cause of a great deal of damage to fruits (Smith, 1889). A source of infection, common in completely ripened fruits and not common to green fruits, is through wounds caused by the cracking of the plums. This cracking is due either to excessive rainfall after a dry period, causing a rapid increase in turgor with the consequent splitting of the fruit, or to water remaining between plums which are in contact. This effect was also noted when ripe plums kept in a moist chamber cracked where they were in contact with the glass if water was present.

The second reason for the ripe-rot effect is the fact that the ripe fruit of some varieties is much more susceptible to rot after infection takes place than the green ones (see p. 388).

VARIETAL RESISTANCE OF PLUMS TO THE FUNGUS

That plums and peaches vary in their resistance to brown-rot has been noted from time to time. This power of resistance has been ascribed to various causes, such as a thick skin in certain varieties of resistant plums, a small amount of down on resistant peaches, and late ripening of some varieties, with consequent avoidance of the disease because of temperature conditions.

During the summer of 1913 attempts were made to determine whether definite differences in resistance to the brown-rot fungus really exist in plum varieties. Inoculation tests were started as early as June 14, when the plums were about one-third grown, and carried through on some varieties until maturity. Infection was brought about at first by spraying the plums with distilled water containing the spores. Later, a more effective method was found to be that of placing the plums in contact with moistened mummies well covered with spores. In both cases the experiments were carried on under bell jars in the laboratory.

RELATIVE RESISTANCE OF VARIETIES

Table IV shows the relative resistance of varieties as determined by the inoculation of 262 plums through uninjured skin and the subsequent rotting of the fruits.

The skin and flesh descriptions, except where indicated, were taken from a table prepared by Dr. M. J. Dorsey, of the Minnesota Experiment Station, in a study of "fruit characters" in hybrid plums, prepared independently of the investigations on resistance. The descriptions of varieties indicated by an asterisk (*) were made by the writer.

TABLE IV.—*Texture of flesh and skin, ripening date, and relative resistance of varieties of plums to Sclerotinia cinerea*

Variety.	Date of ripening.	Texture of flesh.	Texture of skin.	Thickness of skin.	Relative susceptibility.[a]
A × W 2	Aug. 25	Medium firm, tender	Medium	Medium	
A × W 11	Aug. 19	Firm, medium tender	Tough	Thin	++
A × W 12					+++
A × W 15	Sept. 2	Firm, tender	Tough	Medium	++
A × W 17	Aug. 18	Tender	Medium	do	
A × W 18	Sept. 2	Medium firm, tender	Tough	Medium+	+
B × W 1	Aug. 31	Soft, tender	Medium	Medium	++
B × W 2	Aug. 31	do	Tender	Thin	+++
B × W 4	Sept. 2	do	Medium	Medium	++
B × W 5	Sept. 2	Medium firm, tender	do	Medium+	++
B × W 6	Aug. 22	Firm, tender	Tough	Medium	++
B × W 9	Aug. 31	do	do	do	+
B × W 12	Aug. 18	do	do	do	++
B × W 15	Sept. 2	Very firm, medium tender.	Medium	Thick	+
B × W 16	Aug. 27	Soft, tender	do	do	++
B × W 21	Aug. 19	Firm, tender	Tough	do	++
*S. D. No. 1	Aug. 15	Soft, tender	Tender	Thin	++++
*S. D. No. 2	Aug. 15	Firm, tender	Medium	Medium	+
*S. D. No. 3	Aug. 15	do	do	do	+
Burbank	Aug. 17	Soft, tender	Tough	do	+++
Wolf	Sept. 1	Medium firm, tender	Medium	Thick	++
*Ocheeda			Tough	Medium	++
*Harrison			do	do	++
*Surprise			Medium	Thick	+++
*Hammer			do	Medium	+++
*Newman			do	do	+++
*Manitoba No. 1.			Tender	do	++++
*Americana seedling No. 1.		Firm, tough	Tough	Thick	+
*Americana seedling No. 2.			do	Thin	++++
Etopa		Soft, tender	Tender	do	++++
Opata	Aug. 17	do	Medium	do	+++
Okiya	Aug. 18	do	Tender	do	++++
Wakapa	Aug. 18	do	do	do	++++
Compass	Aug. 15	do	do	do	++++
Sand cherry	Aug. 10	do	do	do	++++
*Reagan	Sept.	Very firm, medium tender.	Tough	Thick	+

[a] + Indicates least relative susceptibility; ++++ indicates greatest relative susceptibility.

The results show striking differences in resistance of the several varieties to infection. In the case of very susceptible varieties, as the Compass and sand cherry, it is always very easy to get a large number of infection spots. In the case of a very resistant variety, such as B×W15, it is often very hard to cause infection. In one trial, begun on July 8, 1913, in which green plums, about three-quarters grown,

were inoculated by contact with mummies in a moist chamber, the following results were noted after 27 hours:

Variety.	Number of plums.	Points of contact.	Number of infection spots.
B × W16	1	1	Many.
B × W2	1	1	Do.
Burbank	1	1	20.
B × W15	4	6	None.
Topa	1	1	1.
Opata	1	1	Many.

Another trial with B×W15, directly following this and carried on under the same conditions, showed a few infection spots in three out of five contact points, indicating that in some cases the fungus can enter these resistant plums. A number of other experiments, comparing the relative resistance to infection of B×W15 with that of other varieties, showed results comparable to those given above.

Soon after infection takes place a small decayed spot appears on the surface of the plum. These spots increase in size rapidly in the susceptible varieties and soon completely cover the plum. This often requires not longer than 24 hours after infection has taken place. On the resistant forms, however, the spots increase in size slowly, sometimes taking several days before they entirely cover the plum. The rapidly rotting plums take on the characteristic brown color of rotten fruit; but the slower rotting varieties often become dark blue and when completely rotted become black.

Usually when the susceptible varieties are one-half to three-quarters rotted, they begin producing tufts of chlamydospores over the rotted area. On the sand cherry and some of the sand-cherry hybrids, which are very susceptible, the spore tufts are usually large and numerous (Pl. XXXVIII, fig. 9). Varieties such as B×W21, which appear intermediate in the rapidity with which they rot, usually produce spore tufts, but they are nearly always smaller and less numerous than those on the susceptible varieties (Pl. XXXVIII, fig. 7 and 8). In the case of the most resistant varieties it is seldom that spores are produced if the skin has not been broken. If the plum has been wounded, spores are usually produced through the wound (Pl. XXXVIII, fig. 6). Under particularly favorable conditions pustules may appear through the uninjured skin, in which case they are usually small, and few in number.

RELATION OF SKIN THICKNESS TO RESISTANCE

In order to determine the part played by thickness of skin in resistance, inoculations were made by cutting off a small piece of skin and planting

the spores on this freshly cut surface of the plum in a drop of water. The plums were kept in a moist chamber. The same relative differences in rapidity of rotting were noted in these cases as when the infection took place through the uninjured skin, indicating that mere thickness of skin is not the deciding factor in resistance, as the cells underlying the skin show the same relative resisting powers.

However, it will be seen by referring to Table IV that the varieties which are the most susceptible are the thin-skinned, tender-fleshed ones, while the more resistant varieties are thick-skinned and of a firmer, tougher texture. An examination of prepared slides of the skin of the different varieties confirms these observations, in that all of the very susceptible varieties have a thin skin (Pl. XXXVII, fig. 4), consisting of one or two layers of cells besides the epidermis; while the resistant varieties all have a very thick skin (Pl. XXXVIII, fig. 4), consisting of from five to eight layers of cells. The varieties appearing to be intermediate in resistance have skins varying in thickness, but in all cases examined they are thicker than the susceptible forms. It would seem, then, that there is a rather close correlation between skin thickness and resistance to the brown-rot fungus.

RELATION OF STOMATA AND LENTICELS TO RESISTANCE

In studying the method of infection, a comparison of the stomata and lenticels of the different varieties revealed some interesting and important facts relating to resistance. The lenticels described above, in which no change other than the production of a few flat cells lining the cavity (Pl. XXXVII, fig. 1) took place, were found only in the thin-skinned varieties, as Gold and some of the sand-cherry hybrids. Those in which the lining cells became corky (Pl. XXXVII, fig. 3) were found in the thicker skinned varieties.

In two of the most resistant varieties, B × W15 and A × W9, the formation of lenticels, due to filling of the stomatal cavity with parenchyma cells, was very common (Pl. XXXVII, fig. 4). This condition was not entirely confined to these varieties, as instances were found in many others of the thick-skinned varieties and also in such a thin-skinned variety as Gold (Pl. XXXVII, fig. 2 and 4), where, however, only a few cells were formed that did not in any case completely fill the cavity (Pl. XXXVII, fig. 2).

That the complete plugging of the stomata is a factor in resistance is shown by the fact that many instances were noticed in which these stomata were completely covered by germinating spores, with no resulting infection. It did take place, however, through stomata the cavities of which were only partially filled with these cells and also through those in which only the corky tissue was present (Pl. XXXVIII, fig. 1, 2, and 5). This may explain why it was possible to obtain so few infections in A × W9 and B × W15, even when their surfaces were covered with germinating spores.

PHYSIOLOGICAL RELATION OF FUNGUS TO HOST

That resistance is not entirely due to the partial inability of the fungus to gain entrance to the tissues of the resistance forms is shown by the difference in rapidity of rotting after infection has taken place. A study of the further penetration of the fungus in the resistant and susceptible forms was therefore undertaken.

Previous investigators do not agree as to the manner in which the fungus penetrates the host tissues, some holding that it penetrates the cell walls wherever it comes in contact with them and that it shows no particular affinity for the middle lamella (Cooley, 1914), while others hold that the fungus follows the middle lamella and may or may not split it completely (Schellenberg, 1908; Bruschi, 1912).

The method used in the present study of the relation between the host and the fungus cells was the same as that used in the determination of the method of infection—i. e., a study of prepared slides of infected plum and apple tissue. The stains already mentioned were used. The material consisted of small blocks of plum and apple tissue cut from the edge of the rotting spots and also blocks cut from plums which had been infected within 12 to 30 hours of the time of killing. For this study of the penetration of the fungus, over 220 slides were prepared from material collected from 17 varieties of plums and 4 varieties of apples. In 80 of these slides the fungus hyphæ were clearly differentiated from the host tissue.

PENETRATION

In all cases the fungus shows a very strong affinity for the middle lamella (Pl. XXXVIII, fig. 2, and XXXIX, fig. 1, 2, 5, and 6). No instances were found where the hyphæ had actually pierced the cell walls and entered the cell cavity, so that it seems certain that the hyphæ of *S. cinerea* are unable to penetrate the cell walls of the plum and apple fruits. No record has come to notice of other investigators having extracted from the brown-rot fungus a cellulose-splitting enzym which has the power of dissolving the plum cell walls. Furthermore, that such an enzym is not produced by the fungus in the host tissues is clearly demonstrated by the fact that in completely rotted plum tissue (Pl. XXXIX, fig. 5) and in sclerotia which have been buried in the ground for over 18 months and have produced apothecia, the cell walls are still intact.

From the appearance of the infected tissue it is evident that the fungous hyphæ secrete a substance which splits out the middle lamella slightly in advance of its penetration through the tissue (Pl. XXXIX, fig. 1, 2, 3, 5, and 6). Eventually the middle lamella is completely dissolved, leaving the cells in the rotted area entirely free from one another. Instances comparable to those illustrated were found in nearly all of the slides examined.

The killing of the host cells, so far as is revealed by the microscopical examination, seems due principally to a modification of the osmotic relations of the cells as a result of the disappearance of the middle lamella and to much of the liquid contents of the cells being withdrawn by the fungus to be used in its development. In the plum the chloroplasts and chromoplasts contained in the cells lying directly under the epidermis appeared not to be disintegrating in those cells which had not so collapsed as to make observation impossible. The cytoplasm of the deeper-lying cells was very scant, but showed evidences of plasmolysis, often unmistakably in advance of the penetration of the hyphæ (Pl. XXXIX, fig. 3).

MIDDLE-LAMELLA SOLVENT

The nature of the substance secreted is not at all clear. From the effect on the host tissue it would appear that the middle-lamella-dissolving enzym pectinase was produced, but attempts to isolate it were without success.

Juice was pressed from rotten portions of apples and loquats (*Eriobotrya japonica*) infected with the brown-rot fungus. This was filtered under sterile conditions, in some cases through coarse, and in others fine filter paper. Slices of healthy apple and loquat fruits were partially immersed in the liquid, but showed no softening effect in any case after several days. Further trials with a method to be described later, used in separating pectinase from *Penicillium expansum*, also gave negative results with *S. cinerea*.

In another case a partially rotted apple plug was put into a test tube on cotton above commercial formalin so that the plug did not come in contact with the liquid. It was thought that the fungus would be killed by the fumes, but that if a pectinase were present it would continue to rot the tissue. No further rotting took place, and at the end of five days the tissue, unaffected at the beginning, was still firm and of normal color.

An attempt was made to isolate the enzym pectinase from a culture of *S. cinerea*, 86 days old, on apple cider. The method used was that described by Pringsheim (1910), which consists, in brief, of thorough drying of the material with acetone, followed by pulverization of the dried material and extraction of the enzym with a small quantity of water. On May 8, 1915, succulent twigs of B × W21 plum, sand cherry, and pear (*Pyrus betulifolia*) were partially immersed in the liquid extract in test tubes; also pieces of ripe apple the flesh of which was slightly mealy, and pieces of young peaches, one-quarter grown, were entirely immersed. The tubes were placed in a constant-temperature oven at 35° C. Checks were run, using water in place of the extract.

After 24 and 48 hours the plum, pear, and sand-cherry twigs showed no effects from the treatment other than a slight wilting. The tissues were not softened. The blocks of green-peach fruit showed no softening. After 15 hours the apple plug had softened slightly over the surface, but

was still firm in the center. After 48 hours it had softened completely. A portion not immersed in the liquid, but which came in contact with it at one point, was softening from this point and becoming discolored. The checks in water remained firm and were not discolored.

Although the effect of the extract on the apple tissue appeared to be that of a pectinase, it can hardly be concluded that this enzym was present, as the fruit used was overripe and slightly mealy, and could very easily have been broken down by other solvents contained in the extract.

DeBary (1886) considered the possibility of oxalic acid being the toxic substance produced by *S. libertiana*, because he found the hyphæ often coated with crystals of it; however, he later discarded this notion for the reason that solutions of oxalic acid did not give the same effect as the fungus. Smith (1902) extracted a substance from *Botrytis cinerea*, which, whether boiled or unboiled, caused a rot of the host tissue identical with that caused by the fungus. He concluded it was not an enzym, but that the effect might be due to oxalic acid, which he found to be present in quantities often as high as 2 per cent. Peltier (1912) confirmed the results regarding this action of the extract, but was unable to detect the presence of oxalic acid, even in old cultures.

The possibility of oxalic acid being the toxic substance of *S. cinerea* was considered, as Cooley has demonstrated that it is produced in appreciable amounts in cultures of *S. cinerea* on plum and peach juice, and in peaches which had been rotted by the fungus. In order to determine the effect of oxalic acid on vegetable tissue, small blocks of onion, potato, tomato, dahlia, radish, coleus (young shoot), tomatoes (young shoot), loquat (fruit), canna (bulb), oxalis (petiole), geranium (young shoot), and apple were immersed in 0.015, 0.062, 0.125 per cent solutions of oxalic acid and the effect noted at the end of 24 and 48 hours. In all of the solutions the apple, loquat, and oxalis softened, while in the 0.125 per cent solution only the onion and tomato softened slightly. The potato did not soften even in 0.25 per cent solution. In all cases bleaching occurred. An examination of the different tissues showed that the softening was due to the solution of the middle lamella.

The fact that oxalic acid even in such dilute solutions readily softened the tissues of the apple and loquat, upon both of which the brown-rot grows readily, might indicate that the oxalic acid was the toxic substance, but the bleaching effect produced by the acid and the fact that when used even as strong as 0.25 per cent it had no effect on potato, upon which the fungus also grows readily, would seem to indicate that this acid is not the sole toxic substance produced.

COMPARISON OF FIRM-ROT AND SOFT-ROT

Cooley (1914) pointed out the very interesting fact that, although *P. expansum* and *S. cinerea* apparently acted differently on their hosts, the one producing a soft-rot of fruits and the other a firm-rot, in culture

they gave identical results when grown on media containing cellulose, from various sources, or calcium pectinate. They were able in certain cases to hydrolyze the cellulose, but showed no dissolving action on calcium pectinate.

In order to determine the difference between a soft-rot and a firm-rot caused by fungi which physiologically were acting alike in culture, apples rotting from *P. expansum* were examined. A smear of the rotted tissue revealed the fact that the host cells were entirely separated from one another, but that the walls were apparently intact. A few very small hyphæ could be seen, seeming to be entirely intercellular. Further examination of prepared slides of material, taken both from the oldest portion of a spot 3 inches in diameter and from the edge of the rotting spot, confirmed the above observations. The middle lamella was completely split out between all of the cells in the rotted area, and the cellulose walls were entirely intact. The few very small hyphæ that were found were intercellular (Pl. XXXIX, fig. 4). So far as could be seen, the two fungi, *S. cinerea* and *P. expansum*, act in exactly the same way on the host tissue. The reason for one causing a firm-rot and the other a soft-rot is not, then, due to any differences in physiological action, but appears to be merely mechanical, due to the fact that *S. cinerea* completely fills the intercellular space produced by the collapse of the cells (Pl. XXXIX, fig. 5), with very large hyphæ, while *P. expansum* produces few small hyphæ, which give little support to the host tissues, and, as a consequence, they collapse as the rot proceeds (Pl. XXXIX, fig. 4).

The complete solution of the middle lamella in tissue rotted by *P. expansum* would seem to indicate the presence of a middle-lamella-dissolving enzym. To test this, squares of very fine-grained filter paper were laid on blocks of apple and small portions of flesh from the edge of the rotting spot were laid on the filter papers. All precautions were observed, in order to keep the materials sterile. It was thought that if a pectinase were present it would filter through the paper and cause a soft-rot of the fruit. The papers bearing the rotted flesh were removed after 3½ hours. In four cases out of seven, infection took place through the filter paper and the normal soft-rot followed, while in the three other cases the blocks became soft and translucent at the end of two days, but showed no signs of infection. A microscopic examination showed the cells to be separated from one another, owing to the complete solution of the middle lamella. The checks remained firm. A small portion of the tissue, which rotted in the absence of hyphæ, when transferred to the checks caused them to rot rapidly. This and the fact that in the typical rot spots the middle lamella is completely dissolved in the presence of very few hyphæ would indicate that *P. expansum* secretes a very active middle-lamella-dissolving enzym, pectinase.

RESISTANT AND SUSCEPTIBLE VARIETIES

The fungus hyphæ of *S. cinerea* in both resistant and susceptible fruits show practically no constant differences. In both cases they are large and densely protoplasmic over their entire length. In a few instances hyphæ in resistant forms appeared more knotted and irregular than in susceptible ones, but this could be explained in those cases by mechanical pressure of the small cells of the hypodermal layer, which in the resistant plums appear to be less easily collapsed than in the susceptible varieties. Considerable difference, however, could be noticed in the rapidity with which the hyphæ developed in the two forms. The hyphæ in the susceptible varieties usually completely filled the intercellular spaces as the rot spread, while in the resistant ones fewer hyphæ were produced. A few instances were noticed in resistant varieties of cells lying completely or nearly completely surrounded by hyphæ from which the middle lamella had not been dissolved. This and the fact that in these forms the middle lamella seldom appeared to be dissolved out far ahead of the penetration of the fungus lead to the conclusion that this partial resistance is due to the inability of the toxic material secreted to dissolve the middle lamella as rapidly in the resistant as in the more susceptible varieties, owing possibly to very slight differences in its composition.

That there is an actual difference in the composition of the middle lamella material seems fairly certain. It is well recognized that varieties of plums, apples, and other fruits and vegetables vary greatly in the time required for cooking. Some remain firm after a long period of boiling, while others soften and become mushy after very short heating. An examination of boiled-apple tissue which had become soft revealed the interesting fact that the softening was due in part to a separation of the cells as a result of the middle lamella having been dissolved. The cell walls appeared not to be ruptured at all. In those varities which do not become soft on boiling it is assumed that the middle lamella material is less soluble and therefore is probably of a slightly different chemical composition. It is recognized, of course, that the dissolving action of the fungus upon the pectic substances and solution by hot water are entirely different processes and, therefore, resistance to the fungus and firmness after cooking may or may not be correlated.

In view of the fact that eventually in both resistant and susceptible forms the middle lamella is completely dissolved, the difference in sporulation (Pl. XXXVIII, fig. 6, 7, 8, and 9), as described above, could hardly be explained by variations in middle lamella composition, but rather points to a small amount of some toxic substance being produced either by the host cells or fungus hyphæ, which is not enough to completely stop the growth of the fungus, but merely to retard slightly its normal functioning.

TOXICITY OF ORGANIC ACIDS TO THE FUNGUS

In a series of tests carried on by the writer to determine the relative toxicities of the fruit acids to *S. cinerea*, results were obtained with regard to oxalic acid which may throw some light on the cause of these differences in sporulation. Hanging-drop cultures containing large numbers of the chlamydospores in suspension in solutions of oxalic, tannic, gallic, tartaric (inactive), malic, and citric acids were used. In all of the tests the oxalic-acid solutions were found to be by far the most toxic. As has been noted, Cooley (1914) found this acid to be produced in appreciable quantities by the fungus in culture. In view of this, it is very possible that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphæ to actually become toxic to them, resulting in the production of few or no spore tufts.

RIPE-ROT

The discussion of the penetration of the fungus thus far has had special reference to green and ripening plums, but not to those plums which have begun to soften slightly as a result of the ripening process. It is when the plums begin to soften that the fungus works the greatest havoc, and it is then that variations in resistance are most noticeable in the orchard.

Cook and Taubenhaus (1912) were able to demonstrate a positive correlation between the decrease in the oxidizing-enzym content of the fruits of many plants, due both to maturing and to removal of the fruit from the plant, and a decrease in their resistance to certain diseases. They could show no correlation between acid content of apples and pears and resistance to disease. Cooley (1914) was able to confirm these latter results in the plum, finding that as the plums matured the acid content increased until it reached its maximum at the time of ripening of the fruit, which was also the period of greatest susceptibility to the brown-rot fungus. As acidity will not explain the decrease in resistance of plums to the rot on ripening, can it be explained by a decrease in the oxidizing-enzym content of the plums?

Ripe fruits of the Reagan plum, which is a resistant variety, were sent to this Station from New York on October 22, 1914. On November 7 they were inoculated with the brown-rot, both by spraying on spores and by laying the plums in contact with moistened mummies. By this time the oxidizing enzym should have entirely disappeared, owing both to ripening and to removal from the tree. In spite of this, the plums were found to be still very resistant both to infection and to rot after infection occurred. It is evident then that resistance can not be due in this case to the presence of the oxidizing enzym.

Material of these plums was sectioned, and it was found that in the healthy tissue of these very ripe plums the middle lamella was still

present (Pl. XXXVII, fig. 6). The plums at the time of preserving the material (Nov. 7, 1914) were firm. An examination of the healthy tissue of ripe susceptible varieties revealed the fact that the middle lamella in these was completely dissolved (Pl. XXXVII, fig. 5). These plums were soft when the material was fixed. That the pectic-acid compounds change to pectin in the ripening fruit is a well-known fact. In view of the fact that the brown-rot can only spread after the middle lamella has been dissolved, the reason for the increase in susceptibility on ripening in those varieties which become soft as a result of the normal loss of the middle lamella owing to ripening is readily seen.

The reduced possibilities of infection owing to the plugging of many of the stomata, the causes of which have already been explained, and the persistence of the middle lamella after ripening, as shown by the fact that the fruits remain firm, explain the resistance to brown-rot of such varieties as Reagan, B×W15, B×W9, S. D. Nos. 2 and 3, and Americana Seedling No. 1.

RELATION OF TANNIN CONTENT OF THE HOST TO RESISTANCE

A great deal of attention is being given to the relation between chemical substances within the host cell and resistance. The work of Comes (1913) on the correlation between the increased acid content in wheat plants and rust resistance has been mentioned. Cook and Taubenhaus (1911) were able to show that tannin, a very common product in plants, was toxic in varying degrees to many fungi in culture and considered that it might be a very important factor in resistance. Bassett and Thompson (1911) showed that apples and pears contain an oxidizing enzym capable of producing from gallic acid a tannin-like substance having the power of precipitating protein from solution. They found this product to be toxic to "a fungus." The juices of green apples, pears, and walnut hulls (unboiled) produced a substance which on standing precipitated soluble protein from the juice. They considered this to be a tannin-like substance and to be controlled by the oxidizing enzym.

If the tannins disappear on the ripening of the fruit, as is generally supposed, we may have an explanation of the greater susceptibility of some fruits to disease on ripening. The evidence of the disappearance of tannin on ripening, however, is not at all conclusive. One of the most striking instances of its apparent disappearance is that of the persimmon (*Diospyros virginiana*), the green fruits of which are very astringent, while the ripe, soft fruits are not at all astringent. Gore (1911), however, showed that the tannin did not disappear, but was inclosed in sacs which broke readily in green fruits in contact with saliva, but were not affected in the ripe fruit. Similar structures have been observed in the carob-bean pod (*Ceratonia siliqua*) and in the date fruit. Bassett and Thompson (1911) demonstrated that "apples that had fallen from

the tree showed about twice as much tannin as those freshly plucked." It is a matter of common observation that some plums, especially the sand cherry, contain considerable amounts of an astringent substance, probably tannin, even when dead ripe. It is not altogether clear, therefore, that the disappearance of the tannin on ripening is a cause of the increased susceptibility of ripe fruits to rot.

There is still the possibility that differences in resistance of varieties may be due to unequal tannin content. In order to determine this point, tannin determinations were made of the fruit of 11 varieties of plums. The method used was Proctor's modification of Lowenthal's method as described by Leach (1913, p. 370). The results given in Table V are for tannin substances calculated as gallotannic acid. The determinations were made on fruit which had been picked 14 hours, except in the case of the sand cherry and Compass, which were made directly after picking.

TABLE V.—*Tannin content of ripe and green plums on August 6, 1915*

Variety.	Condition.	Date of ripening.	Percentage of tannin in pulp.	Percentage of tannin in dry matter.	Percentage of dry matter.	Relative susceptibility.
Sand cherry	Ripe	Aug. 1	2.087	15.081	13.84	+ + + +
131 × (sand-cherry hybrid).	...do	.....	.234	1.483	15.75	+ + + +
Compass × pin cherry	...do	.....	.338	2.388	14.17	+ + + +
Sapa	Turning	Aug. 17	.362	3.367	10.75	+ + + +
Compass	Green	Aug. 15	.483	4.229	11.42	+ + + +
A × W12	...do	.....	.482	3.418	14.10	+ + +
Opata	Turning	Aug. 17	.733	4.618	15.87	+ + +
Burbank	Green	...do	.185	1.516	12.20	+ + +
B × W21	...do	Aug. 19	.773	5.777	13.38	+ +
A × W15	...do	Sept. 2	1.131	9.520	11.88	+ +
Americana Seedling No. 1.	...do	.....	.665	3.873	17.17	+

The relative-susceptibility determinations were made at the same time as the tannin determinations and are confirmed by previous tests on some of the varieties and by field observations on all of them.

It is readily seen that very little relationship exists between tannin content and resistance to the brown-rot fungus. Even though a correlation could be shown between tannin content and resistance, it still remains to be proved that the tannin is an actual factor in resistance, since the following facts indicate that it does not come into direct contact with the fungus hyphæ. The hyphæ are apparently always intercellular, and according to Haas and Hill (1913, p. 192)—

> In the cell the tannin occurs in solution in the cell sap, and since tannin forms a precipitate with albuminous matter it follows that the layer of protoplasm around the tannin vesicles must be impermeable to it; if this were not so the protoplasm would be tanned on the production of tannin.

CONCLUSIONS

(1) The brown-rot fungus in Minnesota seems to be identical with that found in other parts of this country and with *Sclerotinia cinerea* of Europe. Chlamydospore tufts vary in color from gray to bright ocher. For the production of the ascus stage the sclerotium apparently must be buried in the ground for two winters. Mummies which have hung on the trees for one year are still capable of producing apothecia.

(2) Infection may take place through the uninjured skin at any time during the development of the plum fruit. The hyphæ enter through the stomata and lenticels. Varieties show great differences in resistance to infection, owing to the production of parenchymatous plugs which fill the stomatal cavity and to lenticels made up of layers of corky cells through which the hyphæ are unable to penetrate. Corky cells lining the stomatal cavity merely delay infection.

(3) Varieties show variations in resistance to rot after the hyphæ have gained entrance. Resistance is apparently correlated with (a) a thick skin; (b) the production of parenchymatous plugs which fill the stomatal cavity; (c) the production of corky walls in the lining cells of the stomatal cavity; and (d) firmness of fruit after ripening. There seems to be no relationship between oxidase content of the fruit and resistance or between tannin content and resistance.

(4) Brown-rot is essentially a ripe-rot, affecting the plums most noticeably as soon as they begin to soften slightly as a result of ripening. Varieties which are resistant remain firm on ripening. Softening during ripening is due to the solution of the middle lamella.

(5) The hyphæ of *S. cinerea* in the tissue of plum and apple fruit are entirely intercellular. The middle lamella is dissolved slightly in advance of the penetration of the hyphæ. The absence of the middle lamella in fruits which have softened owing to ripening explains the greatly increased spread of the disease at ripening time. Attempts to demonstrate the presence of the middle-lamella-dissolving enzym, pectinase, in rotting fruits or to extract it from a culture of the brown-rot fungus on apple cider proved futile.

(6) The rot caused by *S. cinerea* is a firm-rot due to the mechanical support of the hyphæ which completely fill the intercellular spaces left by the collapse of the host cell walls. *Penicillium expansum* produces a soft-rot, because of the fact that few hyphæ are produced and, therefore, little mechanical support is given to the rotted tissue, which as a consequence collapses as the rot proceeds. The hyphæ of *P. expansum* are intercellular and produce a substance which dissolves the middle lamella even in the absence of the fungus hyphæ.

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PLATE XXXVII

Fig. 1.—Lenticel in ripe fruit of Sapa plum. The walls of the cells lining the cavity give the staining reaction of cellulose. × 400.

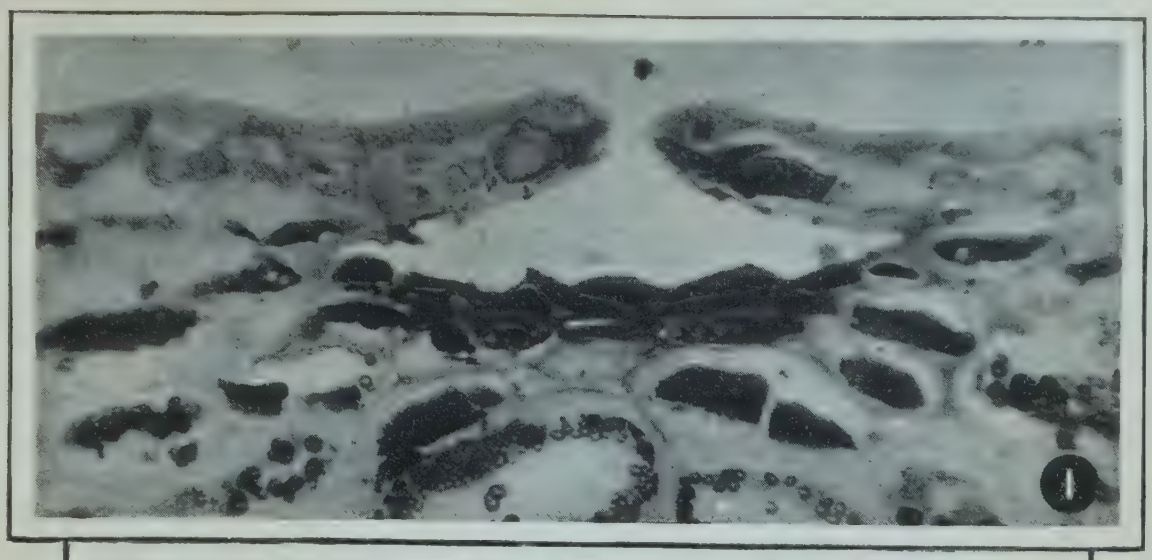
Fig. 2.—Lenticel in ripe fruit of Gold plum partially filled with parenchymatous cells. Infection may take place through a lenticel of this type. × 400.

Fig. 3.—Lenticel in green Burkank plum. The cell walls lining the cavity give the staining reaction of cork. Infection may take place through a lenticel of this type, but only in the manner shown in Plate XXXVIII, figures 1, 3, and 5. × 400.

Fig. 4.—Lenticel in green fruit of B × W 21 completely filled with parenchymatous tissue. Infection can not take place through a lenticel of this type. × 400.

Fig. 5.—Ripe healthy tissue of Sapa plum, showing middle lamella completely dissolved out owing to ripening process. This is the condition found in the ripe fruits of the susceptible varieties. × 60.

Fig. 6.—Ripe healthy tissue of Reagan plum two weeks after picking. The middle lamella is still intact. This is the condition found in the ripe fruit of resistant varieties. × 60.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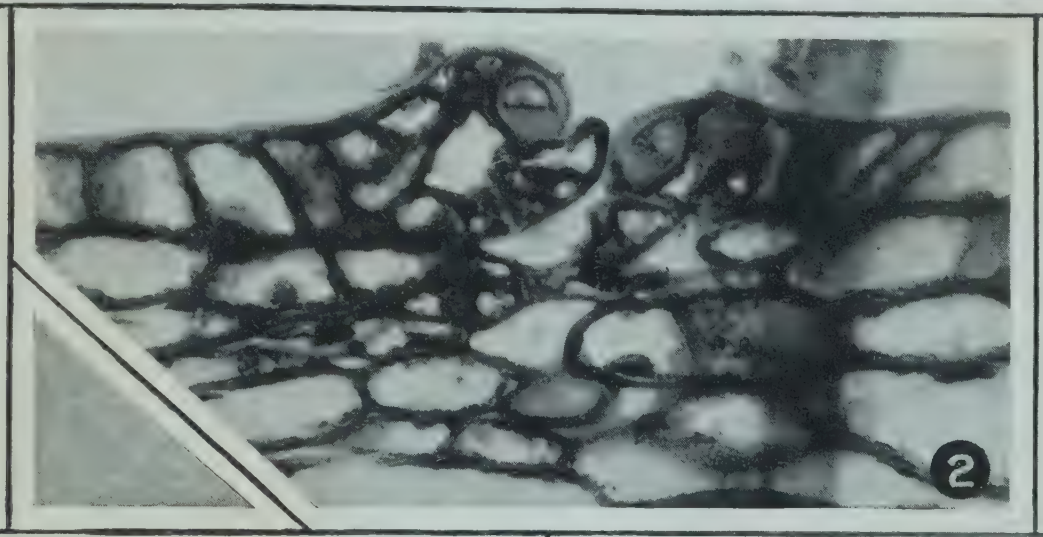
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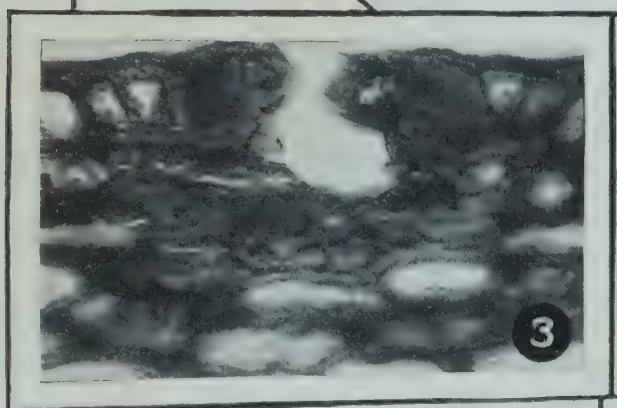
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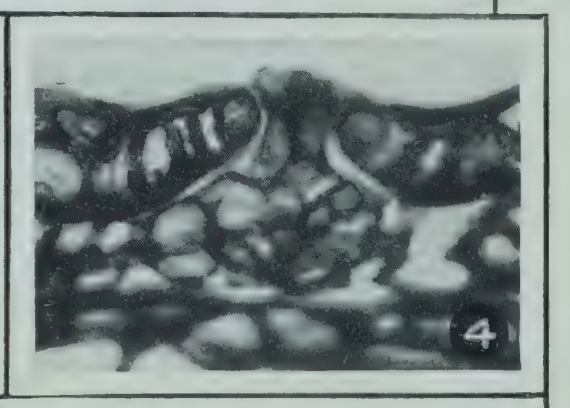
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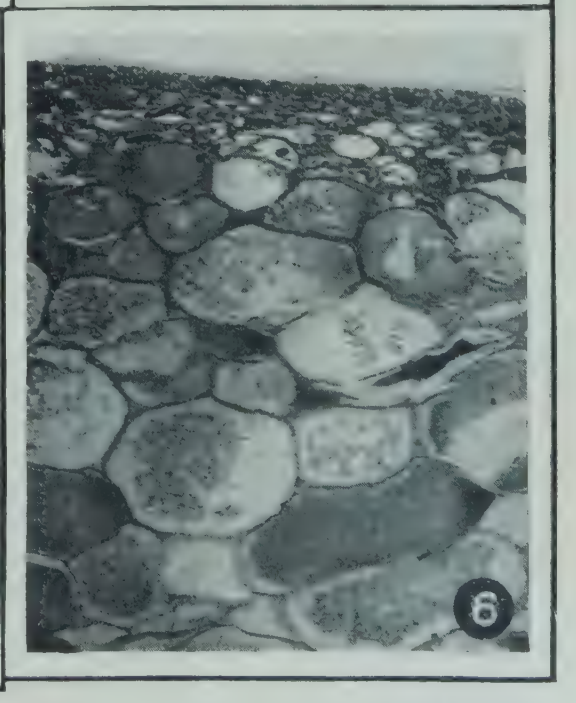
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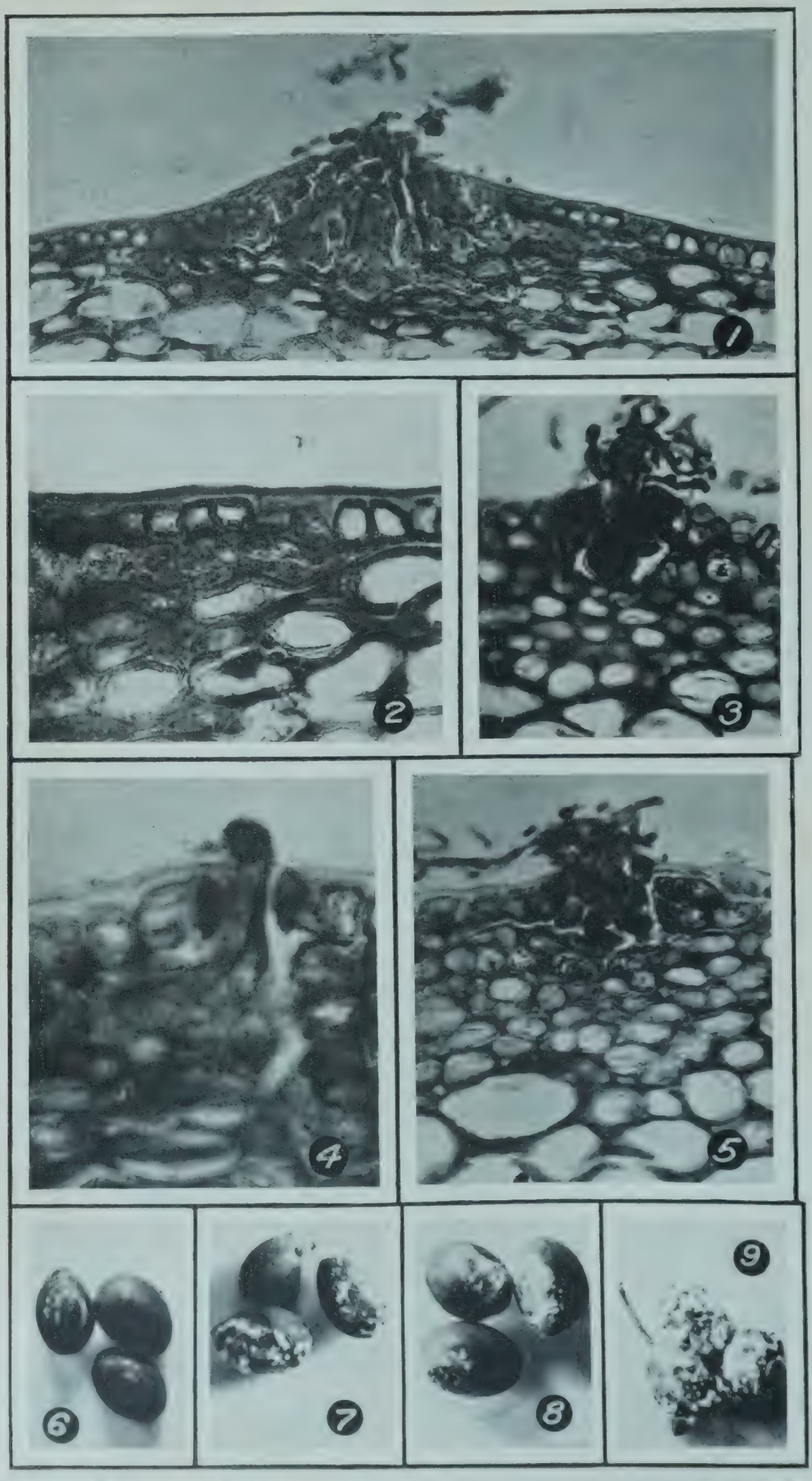
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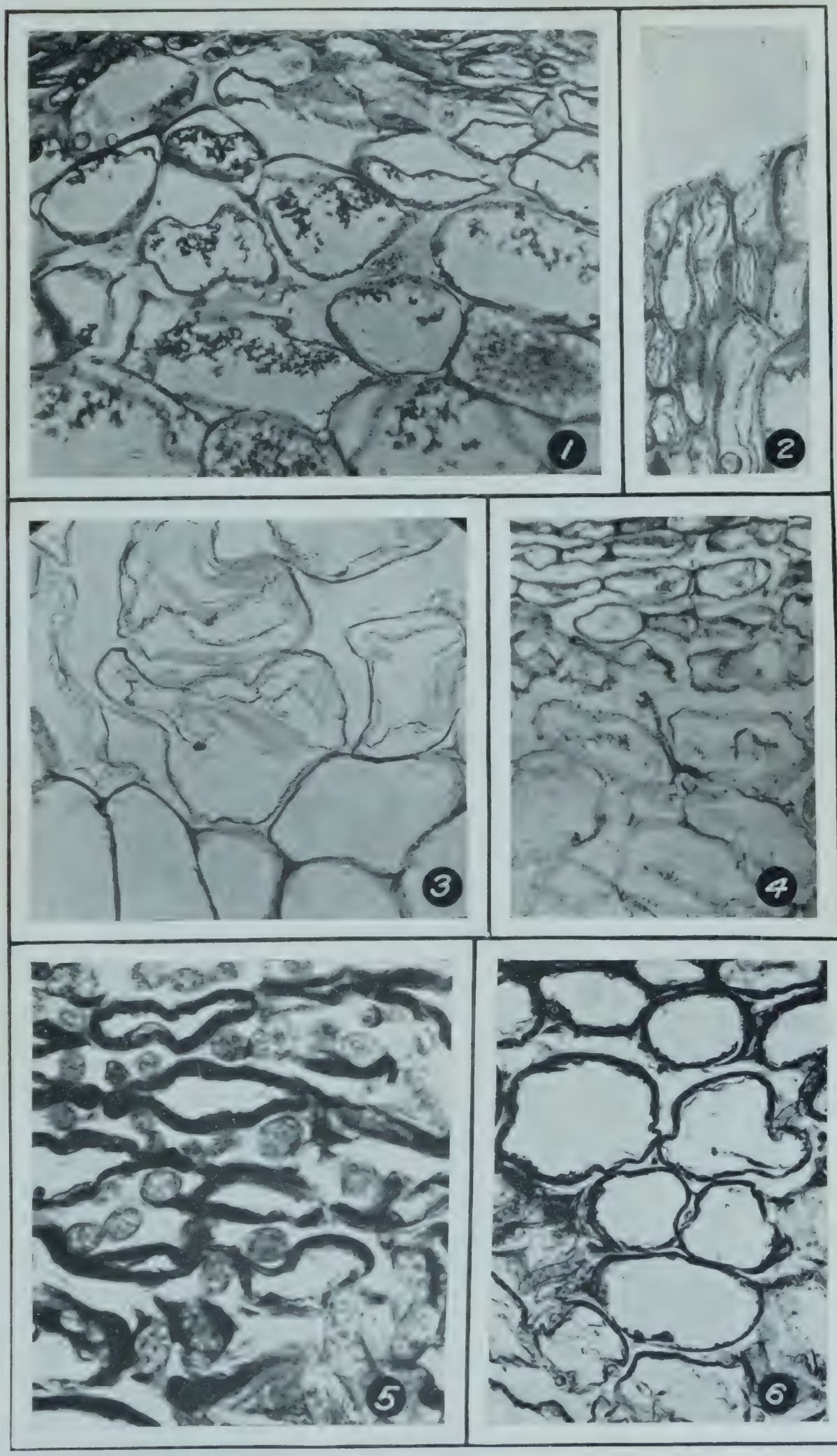
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FREQUENCY OF OCCURRENCE OF TUMORS IN THE DOMESTIC FOWL.[1]

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The work of Rous, Murphy, Tytler, and Lange on the neoplasms of the domestic fowl has aroused some interest in the frequency of their occurrence. In the course of 10 months Rous, Murphy, and Tytler[2] obtained without difficulty about 30 spontaneous tumors in living fowls. On examining 4,000 hens brought to a hotel, Ehrenreich[3] found 7 malignant tumors. All of these occurred in hens more than 1 year old, of which there were 1,000.

For the last 8 years it has been the routine practice at the Maine Agricultural Experiment Station to make autopsies on all birds that either die from natural causes or are killed by accident or for data. In making these autopsies it has been the uniform practice to record the presence of tumors, the organs in which they occur, and whether or not the tumor is of cystic or solid tissue structure. No further study has been made of any tumor. The data were collected primarily because of the possible effect of the presence of the tumor on the other data taken. In going over the records lately, however, their bearing on the frequency of the occurrence of neoplasms in fowls has seemed worthy of analysis and publication. The archives of the laboratory now contain 880 autopsy records sufficiently complete for use in this study.

Of the 880 birds on which autopsies were performed carefully, 79, or 8.98 per cent, had tumors of one sort or another. If we may consider these 880 birds a random sample of fowls as a whole, we may conclude that there are about 90 cases of tumors per 1,000 fowls. While these fowls are not a fair random sample, they are probably nearer one than any other equally large group on which data are at present available. It is possible, however, by the analysis of these records to study the frequency of occurrence of tumors in birds that die from natural causes compared to the frequency in normal birds that are killed. It is also possible to study the relation of the occurrence of tumors to age and sex.

It is a well-known fact that in man there are many tumors which do not primarily affect the health of the host. This seems to be equally true of fowls. Table I shows the occurrence of tumors, first, in birds that

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 86.

[2] Rous, Peyton, Murphy, J. B., and Tytler, W. H. A filterable agent the cause of a second chicken-tumor, an osteochondrosarcoma. *In* Jour. Amer. Med. Assoc., v. 59, no. 20, p. 1793–1794. 1912.

[3] Ehrenreich, M., and Michaelis, L. Ueber Tumoren hei Hühnern. *In* Ztschr. Krebsforsch., Bd. 4, Heft 3, p. 586–591. 1906.

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Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V., No. 9
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either died from or were killed because of disease, and, second, in apparently normal birds accidentally killed or killed for data.

TABLE I.—*Percentage of tumors found in birds dead from natural causes and in normal birds which were killed*

Manner of death.	Total number of birds.	Percentage of birds with tumors present.
Natural causes	660	8.94
Killed	220	9.09
Total	880	8.98

This table shows that there was no significant difference in percentage of tumors found between the two groups of birds. Some of the tumors found in the apparently normal birds were probably early stages of tumors which might later have caused the death of the individual affected. A study of the individual cases of birds with tumors (see Table IV) shows that while in several cases the tumors were the probable cause of death, yet there were many others among the birds which died from natural causes in which the cause of death was entirely unrelated to the presence of the tumor. The close agreement of the two groups in percentage of birds with tumors strengthens the conclusion that in this flock at least there are about 90 cases of tumors per 1,000 birds.

In order to study the influence of age and sex upon the occurrence of tumors, age-frequency distributions were made for each sex. The birds were grouped into half-year classes. There were a few birds whose exact age was not known. These could be classified as "young" (under 2 years) or "old" (over 2 years). The percentage of the birds of each age group which had tumors was then calculated separately for each sex and for the two sexes together. These data are given in Table II.

This table shows that of the 880 birds only 44 were males, while 836 were females. This difference is due merely to the fact that in the adult flocks only a few males were kept (for breeding purposes) and a great many females. It indicates nothing as to the relative morbidity of males and females. Considering the small number of males, it is possible that the apparent difference in the sexes in regard to the occurrence of tumors, 6.82 per cent in the males and 9.09 per cent in the females, may not be significant. A study of the individual cases, however (see Table IV), shows that the organs most frequently affected in the females are the genital organs. It may easily be that on this account there is a real difference in the sexes.

A study of Table II shows that there is a significant correlation between age and the percentage of birds which have tumors. This is also shown in Table III, which is a summary of the data in Table II, combining the

data on all the birds, whether or not their exact ages were known, into two classes, young (under 2¼ years) and old (over 2¼ years).

TABLE II.—*Relation of age and sex to the occurrence of tumors in the domestic fowl*

Age in years (mid-points of class).	Females.				Males.				Males and females.			
	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.
¼	5	81	86	5.81	0	4	4	0	5	85	90	5.56
1	39	424	463	8.42	1	24	25	4.00	40	448	488	8.20
1¼	5	105	110	4.55	1	3	4	25.00	6	108	114	5.26
2	4	60	64	6.25	0	1	1	0	4	61	65	6.15
Total, ¼ to 2¼ years	53	670	723	7.33	2	32	34	5.88	55	702	757	7.27
2½	3	22	25	12.00	0	0	0	.....	3	22	25	12.00
3	5	18	23	21.74	0	1	1	0	5	19	24	20.83
3½	1	1	2	50.00	0	0	0	.....	1	1	2	50.00
4	0	1	1	0	0	1	1	0	0	2	2	0
4½	1	0	1	100.00	0	0	0	.....	1	0	1	100.00
5	0	2	2	0	0	0	0	.....	0	2	2	0
5½	0	0	0	.....	0	1	1	0	0	1	1	0
6	1	0	1	100.00	0	1	1	0	1	1	2	50.00
Total, 2¼ to 6¼ years	11	44	55	20.00	0	4	4	0	11	48	59	18.64
Total, ¼ to 6¼ years	64	714	778	8.23	2	36	38	5.26	66	750	816	8.09
Exact age unknown:												
Young	1	0	1	100.00	0	2	2	0	1	2	3	33.33
Old	11	46	57	19.30	1	3	4	25.00	12	49	61	19.67
Total	76	760	836	9.09	3	41	44	6.82	79	801	880	8.98

TABLE III.—*Summary of the data showing the relation of age and sex to the occurrence of tumors in the domestic fowl*

Age.	Females.		Males.		Males and females.	
	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.
Young (¼ to 2¼ years)	724	7.46	38	5.56	760	7.37
Old (2¼ to 6¼ years)	112	19.64	8	12.50	120	19.17
Total	836	9.09	44	6.82	880	8.98

This table shows that while only 7.46 per cent of the females under 2¼ years have tumors, 19.64 per cent of those over 2¼ years are affected. The result for the males agrees essentially with that for the females, but the number of males is too small to allow us to consider this result as necessarily significant. It is, however, quite certain that the probability of the presence of a tumor in a bird increases as the bird grows older.

The records available for this study show in which organs the tumor is located and whether it is of cystic or solid-tissue structure. These data are given in Table IV.

TABLE IV.—*Data on all the cases of tumors which have been observed at the poultry plant of the Maine Experiment Station, giving their structure and the organs in which they were located*

Autopsy No.	Bird No.	Age.	Sex.	Kind of tumor.	Tumors[a] located in or attached to—															Cause of death.
					Ovary.	Oviduct wall.	Oviduct ligament.	Mesentery.	Abdominal wall.	Intestine wall.	Kidney.	Gizzard.	Liver.	Spleen.	Pancreas.	Heart.	Testis.	Breast bone.	Organ not recorded.	
		Yr. m.																		
491	408G	0 6	♀	Cystic															+	Unknown.
500	466G	7	♀	Tissue					+											Internal bleeding; point not recorded.
504	559G	7	♀	do					+				*	*						Probably abnormal condition of liver and spleen.
12	81D	8	♀	do											+					Killed because collapsed trachea almost prevented breathing.
967	9070	8	♀	Cystic		+														Killed for data.
193	365E	9	♀	Tissue					+											Egg bound; anal opening closed by egg.
199	222E	9	♀	do					+		*		*	*						Probably tumors.
184	656D	9	♂	do					+											Unknown.
222	104E	10	♀	do				+	+					+						Probably tumors.
200	321E	10	♀	do					+	+										Unknown.
109	440D	10	♀	do	+															Digestive disorders.
107		10	♀	do	+															Internal hemorrhage; point not recorded.
344	184F	10	♀	do					+		+		*							Probably tumors.
110	201D	10	♀	do	+(small)															Choked to death.
219	430E	11	♀	do							+		*							Probably tumors.
130	292D	11	♀	do	+						*		*							Internal hemorrhage; point not recorded.
117	260D	11	♀	do	+															Unknown.
147	380D	1 0	♀	Cystic									+							Probably tumors.
143	708D	1 0	♀	Tissue	+	+					*		*							Internal bleeding from tumor on liver.
138	680D	1 0	♀	do	+								*							Possibly tumors.
132	330D	1 0	♀	do	+						*									Do.
127	827D	1 0	♀	do	+(small)															Killed because she was sick. Apparently normal except tumor.
1011	117M	1 0	♀	do			+													Killed because of roup.
1014	371M	1 0	♀	do					+											Catarrh.
869	482K	1 0	♀	Cystic		+														Unknown.

258	341E	1 0	♀	...do				+											Heart and veins filled with clotted blood.
238	75E	1 0	♀	Tissue				+			*		*	*					Do.
520	148G	1 0	♀	Cystic	+						+			+					Probably tumors.
257	457E	1 0	♀	Tissue											+				Hung in trap nest.
260	497E	1 0	♀	Cystic		+													Killed for data.
389	280F	1 0	♀	Tissue								+							Do.
158	521D	1 1	♀	...do	+								*	*					Internal hemorrhage from ruptured spleen.
154	374D	1 1	♀	...do	+								*						Unknown.
152	583D	1 1	♀	...do	+														Internal hemorrhage; point not recorded.
655	87H	1 1	♀	Tissue containing cysts.	+								*						Internal hemorrhage from tumor.
538	16G	1 1	♀	Tissue			+												Unknown.
536	279G	1 1	♀	...do							+		+						Probably tumors.
413	1229	1 1	♀	Cystic		+	+												Gout.
287	22E	1 1	♀	Tissue containing cysts.	+														Internal hemorrhage from tumor.
255	306E	1 1	♀	Cystic	+														Peritonitis. Heart and veins filled with clotted blood.
248	429E	1 1	♀	Tissue							*			*		+			Internal hemorrhage; point not recorded.
235	432E	1 1	♀	...do					+				*	*					Heart and veins filled with clotted blood.
144	88D	1 1	♀	...do									*					+	Digestive disorders.
164	678D	1 2	♀	...do	+														Probably tumors.
168	652D	1 2	♀	...do		+													Digestive disorders. Toxemia.
174	61D	1 3	♀	...do	+				+										Probably tumors.
1047	72M	1 3	♀	...do			+												Died on nest with broken egg partly out.
923	646	1 4	♂	...do												+			Probably tumor.
331	1367 '08 ch.	1 5	♀	Cystic	+								*	*					Internal hemorrhage from liver.
939	30K	1 6	♀	Tissue			+												Digestive disorders.
616	379H	1 7	♀	...do				+	+										Killed because of roup.
508	1233	1 10	♀	...do	+														Internal hemorrhage from tumor.
350	1133	1 11	♀	...do		+													Peritonitis due to eggs in body cavity.
60	678C	1 11	♀	Cystic							+		*						Killed for data.
1017	1548	2 0	♀	Tissue		+		+		+									Probably tumors.
307	1083	2 3	♀	...do			+	+	+	+									Do.
713	1280	2 5	♀	Cystic			+												Killed for data.
810	14H	2 5	♀	Tissue	+														Killed for meat.
69	521B	2 11	♀	...do	+								*						Killed for data.
84	99B	2 11	♀	...do	+														Do.

[a] Asterisk (*), organ hypertrophied probably by infiltration with tumor cells.

TABLE IV.—*Data on all the cases of tumors which have been observed at the poultry plant of the Maine Experiment Station, giving their structure and the organs in which they were located*—Continued

Autopsy No.	Bird No.	Age.	Sex.	Kind of tumor.	Tumors[a] located in or attached to—															Cause of death.
					Ovary.	Oviduct wall.	Oviduct ligament.	Mesentery.	Abdominal wall.	Intestine wall.	Kidney.	Gizzard.	Liver.	Spleen.	Pancreas.	Heart.	Testis.	Breast bone.	Organ not recorded.	
		Yr. m.																		
83	130B	2 11	♀	Tissue ..	+															Killed for data.
414	1074	3 0	♀	...do			+													Congestion of lungs.
296	1067	3 1	♀	...do		+														Do.
908	20H	3 5	♀	Cystic...	+					++										Roup.
952	1229	4 7	♀	...do	++															Killed because of roup.
960	9024	5 10	♀	...do	+															Peritonitis due to egg masses in body cavity.
973	000	Young.	♀	...do		+														Unknown.
185	13A	Old.	♀	Tissue ..	+															Do.
181	8A	Old.	♀	...do	+															Do.
81	187	Old.	♀	...do				+												Killed for data.
45	349	Old.	♀	...do	+			+												Do.
89		Old.	♀	...do	+								*							Do.
68	395	Old.	♀	...do	+								*							Do.
58	50	Old.	♀	...do	+								*							Do.
80	150	Old.	♀	Cystic...	+															Do.
65	152	Old.	♀	...do	+															Do.
94	2	Old.	♀	Tissue ..	+															Do.
93		Old.	♀	...do	+															Do.
954		Old.	♂	...do														+		Probably tumor.
Total.	79		{76 ♀ 3 ♂}		37	10	8	8	13	5	5	1	2	2	2	1	1	1	2	98 tumors.
Total percentage.[b]					37.76	10.20	8.16	8.16	13.27	5.10	5.10	1.02	2.04	2.04	2.04	1.02	1.02	1.02	2.04	

[a] Asterisk (*), organ hypertrophied probably by infiltration with tumor cells.

[b] Percentages are calculated on base of 98 tumors, although they all occurred in 79 birds.

Attention has already been called to the fact that tumors occurred as frequently in apparently normal birds which were killed as in those which died from natural causes. From the data given in Table IV it may be seen that many of the birds with tumors died from diseased conditions apparently not related to the presence of the tumors. There were, however, a number of cases where the size and distribution of the tumors and the condition of the organs to which they were attached indicated that the tumors were the probable cause of death. Associated with many cases of tumors was a hypertrophied condition of the liver, spleen, or kidneys. The liver was most often affected. In fact, 19, or 24.05 per cent, of the individuals having tumors had enlarged and soft, friable livers. In the absence of microscopic examination of these organs, it can not be definitely stated that this hypertrophy was due to infiltration with tumor cells.

Table IV also shows that in several cases the immediate cause of death was internal hemorrhage, either from the tumor surface, the tissue immediately beneath, or the hypertrophied liver or spleen. There were several tumor cases in which death was recorded as due to internal hemorrhage but in which the bleeding point was not recorded. It is probable that in these cases also the bleeding took place either from the tumor or from the hypertrophied liver or spleen.

Our macroscopic examination of the tumors limited their classification to the two groups of tissue tumors, formed of solid masses of tissue or sometimes of large tissue masses inclosing masses of pus, mucus, or clotted blood, and cystic tumors, which were sacs filled with liquid. Table IV shows that 18, or 22.78 per cent, of the tumors observed were cystic, while 59, or 74.68 per cent, were tissue tumors. There were two cases (2.59 per cent) of ovarian tumors where cysts were attached to tissue tumors.

Table IV also shows the organ distribution of the tumors. It should be borne in mind that this is essentially the distribution in females, as only three males are included in the data. The organ most frequently affected is the ovary (37.76 per cent [1] of all the tumors occur in that organ). The oviduct wall and ligament harbored 18.36 per cent—that is, in the female the genital organs are the organs most frequently affected by tumors. The number and percentages for each of the other organs are given in the table. Table IV also shows that in most cases the tumor was confined to one organ. In 15 cases, however, the tumor had undergone metastasis, since tumors of similar sorts occurred in 2 (11 cases), 3 (3 cases), or 4 (1 case) organs. Attention has already been called to the frequent association of hypertrophied livers, spleens, and kidneys with defined tumors in other organs.

[1] These percentages are calculated on the basis of 98 tumors, although they all occurred in 79 individuals

SUMMARY

The purpose of the present paper is to record the data on the frequency of occurrence of tumors in the domestic fowl which have been collected during eight years' routine autopsy work at the Maine Agricultural Experiment Station.

The chief points brought out by an analysis of these data are as follows:

(1) Of the 880 birds autopsied 79, or 8.96 per cent, had tumors. That is, there were 90 cases of tumors per 1,000 birds.

(2) There was no significant difference in frequency of occurrence of tumors between birds which died from natural causes and apparently normal birds which were killed.

(3) There is a significant positive correlation between age and the occurrence of tumors. Only 7.37 per cent of the birds under 2¼ years had tumors, while neoplasms were present in 19.17 per cent of those that were over that age.

(4) In birds with tumors which died from natural causes, the tumors were directly or indirectly the probable cause of death in from one-third to one-half the cases.

(5) There was a decided tendency for the association of hypertrophied (apparently due to cell infiltration) liver, spleen, or kidney with the presence of tumors in other organs.

(6) Death often resulted from internal hemorrhage from the tumor, the underlying tissue, or the hypertrophied liver or spleen.

(7) The tumors can be classified into cystic and tissue tumors; 22.78 per cent of the tumors were of cystic and 74.68 per cent of solid-tissue structure. There were two cases of tissue tumors to which cysts were attached.

(8) In the females[1] the organs most frequently affected were the genital organs; 37.76 per cent of all the tumors being in the ovary and 18.36 per cent in the oviduct and oviduct ligament.

(9) In most cases the tumors were confined to one organ. In 15 cases, however, the tumor had evidently undergone metastasis, since tumors of similar nature occurred in from two to four organs.

[1] Autopsies were made on too few males to yield reliable data.

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., DECEMBER 13, 1915 NO. 11

TRANSLOCATION OF MINERAL CONSTITUENTS OF SEEDS AND TUBERS OF CERTAIN PLANTS DURING GROWTH

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INTRODUCTION

Several years ago it was observed by Dr. J. H. Kastle, Director of the Kentucky Experiment Station, that the morning-glory vine (*Ipomoea purpurea*) after removal from the soil would continue to grow when its roots were immersed in rain water. Often the growth of this vine attained a length of several feet, bloomed, and produced seeds. During this period the lower leaves etiolated, withered, and ultimately dried up. Evidently the new growth attained by this plant under these conditions was largely at the expense of the various materials contained in the roots, the lower part of the stem, and the lower leaves; especially was this true of the mineral matter required by the new growth, inasmuch as no mineral substance was supplied by the rain water. It therefore occurred to Dr. Kastle that it would be of interest to determine the translocation of the mineral matter in this vine under these conditions. Accordingly, a number of morning-glory vines were completely removed from the soil in which they had grown, and the soil was carefully washed from their roots, which were placed in wide-mouth bottles containing distilled water, the vines being trained on strings arranged vertically in a window. Under these circumstances the vines were found to increase in length by several feet. They put out new roots and a large number of new leaves and in many instances bloomed and produced seeds. Unfortunately, with the limited space at our disposal we were unable to secure a sufficient amount of material to determine the translocation of the mineral substances of the plants under these conditions, and it was found necessary to abandon the experiment with the morning-glory for the time being. However,

[1] The writer wishes to acknowledge the many valuable suggestions made by Dr. Kastle during the progress of these experiments.

we are still of the opinion that on account of its hardiness under all sorts of conditions this plant would lend itself better than any other to such studies as those herein contemplated, and we hope to take it up again at some future time.

In thinking over the subject of the translocation of mineral matter during plant growth it occurred to us that it might be of interest to determine the translocation of the mineral matter contained in the seeds and tubers of certain plants during the period of sprouting. Therefore, our present experiments have been confined to the seeds of the garden bean (*Phaseolus vulgaris*), corn (*Zea mays*), and to the potato tuber (*Solanum tuberosum*). Up to this time our work has been confined to the measurement of the translocation of phosphorus, calcium, potassium, magnesium, and silicon.

EXPERIMENTS WITH GARDEN BEANS

The cotyledons of the garden bean were found to contain a considerable amount of mineral matter, and the seedlings of this plant are hardy and well adapted to our requirements. The only difficulties experienced in growing these seedlings under the conditions of these experiments were the growth of molds and the attack of the seedling by the damping-off wilt. The bean in this instance was germinated and allowed to grow to maturity at the expense of the food stored in the cotyledons, extreme care being taken that they should receive no mineral food from external sources. We, of course, realized that the growth of any plant in distilled water is more or less abnormal; yet these beans germinated and produced perfect seedlings with well-developed leaves.

Great difficulty was experienced in keeping down the growth of molds during the process of germination and in preventing the damping-off wilt from attacking the seedlings. In order to overcome these difficulties, every precaution was taken to sprout and grow these seedlings under aseptic conditions. The distilled water employed was boiled for 20 minutes before coming in contact with the beans. The germination and growth of the seedlings were carried out in a dust-proof closet constructed for that purpose. A framework of wood was made and covered inside and out with cheesecloth, leaving an air space of about 2 inches. During the experiment both layers of the cheesecloth were kept moistened with a 50 per cent solution of glycerin and water. This prevented dust and spores from entering the closet; yet it allowed a free passage of air and light. An opening was made in the side of the closet just large enough to admit the head and shoulders of a man. Over this opening was hung a curtain, so arranged as to exclude dust while working inside and when the closet was closed.

The seedlings were never allowed to come in contact with glass. The germinations were made in large porcelain evaporating dishes in which

were placed round perforated porcelain plates, similar to those used in desiccators, on top of which were placed two circular pieces of blotting paper which had been treated with dilute hydrochloric acid and washed free from chlorids with distilled water. Small lamp wicks connected these blotters with the water in the bottom of the dish, so that they would remain moist during the period of germination. Just previous to placing the beans between the blotters the entire apparatus was sterilized by heating at 180° C. for two hours.

The germinated beans were transplanted to test tubes which had been carefully paraffined inside and in each of which was placed a plug of cotton about half an inch from the top and held in place by a small amount of paraffin. The cotton was the purest we could obtain and was treated with dilute hydrochloric acid and washed with sterile distilled water until no test for chlorids could be obtained. This cotton gave practically no ash when incinerated.

In beginning this experiment 1,400 perfect beans were selected, cleaned with a damp cloth, and divided into two lots of 700 each. These lots were labeled "A" and "B," respectively. The 700 beans labeled "A" were placed in a flask and covered with 95 per cent alcohol containing 20 per cent of formalin and allowed to stand for 20 minutes. The beans were then drained and washed free from alcohol with sterile distilled water. The alcoholic drainage and washings were evaporated to dryness and saved for analysis, being labeled "11" in Table I. The beans were now transferred to the sterile germinating dishes described above and placed between blotters, care being taken that the beans did not touch each other. Throughout the germination of the beans sterile distilled water was added in just sufficient amounts to keep the beans moist. Germination started at once, and the small radicle appeared in from two to three days and in some instances was half an inch in length by the end of the fourth day. As soon as this stage was reached, the integuments were removed from the cotyledons with sterile, platinum-tipped forceps, care being taken not to bruise the cotyledons nor allow dust or dirt to come in contact with them. The integuments were preserved and labeled "9" in Table I. The seedlings were then transferred to paraffined test tubes ¾ by 6 inches, the seedlings being held in place with a small quantity of sterile cotton. The test tubes were filled with sterile distilled water, which was replaced as fast as it was removed by the plant or by evaporation. The seedlings began to grow immediately, putting forth roots and plumules. Some of the beans on germinating proved to have imperfect cotyledons; these with a number which had been bruised during the removal of the integuments were discarded, so that at the end of the experiment only 609 seedlings had been allowed to mature. This number furnished the material for analysis.

TABLE I.—*Analysis of separate parts of bean seedlings and whole beans*

(A) SEEDLINGS

Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorus as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.	
		Gm.	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*
1, 218 Cotyledons (exhausted)	7	70. 8239	3. 3441	4. 72	1. 1768	35. 18	0. 0819	2. 45	0. 0959	2. 87	1. 5459	46. 52	0. 0367	1. 10
609 Integuments (removed)	9	16. 5423	. 6372	3. 85	. 0121	1. 91	. 1965	30. 85	. 0469	7. 37	. 1224	19. 22	. 0137	2. 00
609 Roots	8	7. 5516	. 7785	10. 31	. 1916	24. 61	. 0206	2. 65	. 0129	1. 67	. 2993	38. 46	. 0175	2. 25
609 Upper stems	6	7. 1577	. 5611	7. 83	. 1441	25. 69	. 0060	1. 07	. 0097	1. 74	. 2084	37. 16	. 0062	1. 10
609 Lower stems	5	21. 6122	1. 1339	5. 24	. 3740	32. 99	. 0278	2. 45	. 0341	3. 01	. 4224	37. 26	. 0085	. 75
1, 218 Leaves	4	31. 1819	2. 1048	6. 78	. 6064	29. 00	. 0157	. 95	. 0574	2. 73	. 9539	45. 32	. 0210	1. 00
Drain	11	3. 9526	1. 2335	31. 20	. 2026	18. 12	. 0242	1. 96	. 0628	5. 09	. 5996	48. 61	. 0102	. 82
Total weight		158. 8222	9. 7931		2. 7076		. 3727		. 3197		4. 1519		. 1138	

(B) CONTROL BEANS

1, 218 Whole cotyledons	3	169. 3022	7. 3058	4. 31	2. 5000	34. 22	0. 0949	1. 30	0. 2388	3. 27	3. 7038	49. 19	0. 0403	0. 55
609 Integuments	2	16. 4422	. 7369	4. 48	. 0251	3. 41	. 2505	34. 00	. 0424	5. 76	. 1786	24. 24	. 0081	1. 10
Drain	1	2. 7616	. 6354	23. 07	. 0582	9. 16	. 0176	2. 77	. 0237	3. 74	. 2823	44. 44	. 0117	1. 85
Total weight		188. 5060	8. 6781		2. 5833		. 3620		. 3049		4. 1647		. 0621	

As the growth of the seedlings proceeded, the cotyledons began to shrink and finally turned brown. The root development in all cases was good, nearly filling the test tubes, and each seedling developed two perfect leaves. The seedlings were allowed to grow until they began to etiolate and wilt, this period being reached in from 17 to 22 days. The plants thus grown were very uniform in size and development, the average height being 6½ inches. During their development care was taken that they should not touch each other. As fast as they matured, they were removed from the test tubes and the cotton carefully removed from the stem and roots. The plants were then divided into roots (8),[1] lower stems (5) which averaged 4½ inches in height, exhausted cotyledons (7), upper stems (6) which averaged 2 inches, and the leaves (4). The liquid remaining in the test tubes was evaporated to dryness and added to the washings (11).

Six hundred and nine selected beans labeled "B" received the same treatment as those labeled "A," except they were allowed to live only until the radicle had appeared and the integument had softened. The integument (2) and the cotyledons (3) were carefully air-dried, as were the above-mentioned plants. The drainage and washings (1) from these beans were carefully evaporated to dryness. These several parts of the beans were analyzed to check the analyses of the seedlings, the results of which are given in Table I.

In analyzing the separate portions of the air-dried material which had been carefully ashed at a dull-red heat, three portions of 0.2000 gm. each were carefully weighed out. In one portion phosphorus and silica were determined, while in another portion the determination of potassium was made. The methods used were essentially the official methods of the Association of Official Agricultural Chemists.[2] In a third portion of the ash, calcium and magnesium were determined according to the method of McCrudden.[3]

In Table I are to be found the results of the analyses of the separate portions of 609 seedlings and the separate parts of 609 beans.

It is evident from the results given in Table I that the weight of the total ash of the seedlings agrees fairly well with the total weight of the ash of the bean control, the difference being due in all probability to unavoidable outside contamination during the period of growth. The comparative analyses of the inorganic constituents fall well within the limit of experimental error. The greatest difference is observed in the case of silica, the seedlings containing nearly twice as much as the beans.

[1] The numbers in parentheses refer to the number of part in the tables.

[2] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

[3] McCrudden, F. H. The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine, and feces. *In* Jour. Biol. Chem., v. 7, no. 2, p. 83–100. 1910.

——— The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in urine. *In* Jour. Biol. Chem., v. 10, no. 3, p. 187–199. 1911.

This is probably due to unavoidable contamination. It is of interest to note that the integument contains 52.72 per cent of the total calcium oxid found in the bean; it is also interesting to find that the amount of phosphorus and potassium in the integument is very small. It is shown that a marked accumulation of the mineral elements in the leaves and lower stems occurs during growth. This is more clearly shown where the results are expressed as the percentage distribution of the mineral constituents that actually migrated from the cotyledons, as seen in Table II.

TABLE II.—*Percentage distribution of the mineral constituents of bean seedlings*

Part.	Part No.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2)
Cotyledons (exhausted)	1	47.20	54.53	45.67	45.07	40.82
Roots	4	7.68	13.72	6.14	8.72	19.47
Upper stems	5	5.78	3.99	4.62	6.07	6.90
Lower stems	3	15.00	18.51	16.24	12.31	9.45
Leaves	2	24.34	10.45	27.33	27.83	23.46

In the foregoing experiment we have germinated beans, and they have grown until they died from the want of nourishment. From all physical appearances the growth of the seedlings has been normal. This growth has been at the expense of the food material stored in the cotyledons, the carbon dioxid inspired from the air, and the distilled water received through the roots. Every precaution was taken to exclude all mineral matter from external sources. Referring to Table II, it is seen that approximately 50 per cent of the total mineral content of the cotyledons remained unused and that approximately 50 per cent was translocated to different parts of the seedlings during growth. As might be expected, the greatest quantity of these elements migrate to the leaves and the next greatest quantity locate in the lower stems. The large amount of calcium and silica locating in the roots is also of interest.

These results serve to emphasize the importance of the mineral matter both to the seedlings and to the sprouting seed or cotyledon. In other words, it would seem from these results that the mineral matter originally present in the seed or in the cotyledons functions in the act of sprouting in two different ways: First, to promote the enzymic changes occurring in the sprouting cotyledons and seeds themselves; and, in the second place, to support the growth and development of the seedlings. The growth will therefore depend somewhat at least on the total mineral matter originally present in the cotyledons or seeds, a part of this being translocated to meet the requirements of the growing seedling. Approximately an equal part or, at any rate, a relatively large amount of the mineral matter remains in the seed or cotyledon to support and promote those enzymic changes characteristic of the seed or cotyledon in an active katabolic condition.

EXPERIMENTS WITH CORN

Similar experiments have been tried with corn, except that the seedlings were grown in aluminum cups instead of in paraffined tubes. One thousand grains of corn were germinated, transferred to aluminum cups, and allowed to grow for 23 days, when they began to etiolate. During this time these seedlings attained a height of 9 inches. At this point they were removed from the cups and dissected as follows: Leaves (2), exhausted cotyledons (3), stems (4), and roots (5). (See Table III.) These were controlled by the same number of whole corn grains (1) as given also in Table III. These several lots of material were analyzed in the same manner as the bean seedlings. In this experiment we have also followed the translocation of iron and aluminum. Unfortunately, the results obtained with these two last-named elements show contamination from the aluminum cups used in the experiment. The results of the analyses of the ash of corn grain and of the several parts of the seedlings thereof are given in Table III.

It will be seen from the results of these analyses that the sum of the total ash of the several parts of the corn seedling exceeds the total ash of the corn grain by 0.9487 gm. This is doubtless to be explained by the fact that iron and aluminum were taken up in considerable amounts from the cups and also by contamination with small amounts of dust from the outside air. It will be seen that the sum of the amounts of phosphoric acid, potash, and magnesia in the several parts of the corn seedling agrees with that of the corresponding amounts of these substances found in the corn grain, within the limits of experimental error. A point of interest in this connection is that magnesia is greatly in excess of lime in the grain of corn and in the several parts of the seedling obtained therefrom. The amounts of lime, silicon, iron, or aluminum found in the several parts of the seedling are in excess of the amounts of these substances found in the grain. As already pointed out, this discrepancy is doubtless due to outside contamination. Under the conditions prevailing in this experiment approximately two-thirds of the total mineral matter of the corn grain has been translocated to the stems, roots, and leaves of the seedling during the process of growth. It is evident further that approximately the same amounts of this mineral matter go to stem and roots, respectively, whereas a somewhat larger amount of the mineral matter migrates to the leaves of the seedlings. The fact that a relatively large amount of the mineral matter, amounting in this case to something over one-third of the whole, remains in the exhausted cotyledon is of interest and doubtless has the same significance for the growth of the seedling as is believed to obtain in the case of the bean, already discussed. The percentage distribution of the mineral constituents of corn during the growth of the seedling is shown in Table IV.

TABLE III.—*Analysis of separate parts of corn seedlings and whole corn grains*

SEEDLINGS

Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorous as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.		Iron oxid (Fe_2O_3) in ash.		Aluminum oxid (Al_2O_3) in ash.	
		Gm.	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*
3,000 Leaves	2	43.2716	2.0107	4.64	0.7845	39.02	0.0502	2.50	0.1425	7.09	0.6428	31.92	0.0613	3.05	0.0012	0.06	0.0096	0.48
1,000 Cotyledons (exhausted)	3	342.3911	2.7254	.79	1.2858	47.18	.0735	2.70	.2335	8.57	.3597	13.20	.1812	6.65	.0234	.86	.0136	.50
1,000 Stems	4	36.7137	1.3292	3.62	.5186	39.02	.0221	1.65	.0835	6.29	.3808	28.65	.0198	1.50	.0107	.81	.0086	.65
1,000 Roots	5	48.9321	1.3514	2.76	.3849	28.46	.0216	1.60	.0369	2.73	.4153	29.92	.0466	3.45	.0120	.89	.2471	18.29
Total	.....	471.3085	7.4167	.....	2.9738	.....	.1674	.....	.4964	.....	1.7986	.....	.3089	.....	.0473	.....	.2789	.....

WHOLE GRAINS

1,000 Whole corn	1	461.9412	6.4680	1.40	3.0250	46.77	0.0562	0.87	0.4760	7.36	1.8919	29.25	0.0822	1.28	0.0116	0.18	0.0051	0.079

TABLE IV.—*Percentage distribution of the mineral constituents of corn seedlings*

Part.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2).	Iron oxid (Fe_2O_3).	Aluminum oxid (Al_2O_3).
Leaves	26.38	30.02	28.71	35.74	19.84	2.54	3.44
Exhausted cotyledons	43.24	43.86	47.04	20.00	58.66	49.47	4.88
Stems	17.44	13.21	16.82	21.17	6.41	22.62	3.08
Roots	12.94	12.91	7.43	23.09	15.09	25.37	88.60

A comparison of these individual mineral constituents shows that except in the case of potash approximately 50 per cent thereof have been translocated from the cotyledons to the several parts of the seedling during growth, and that the translocation of potash is greatly in excess of that of the other mineral constituents. The taking up of such considerable amounts of iron and aluminum from the aluminum cups in which these seedlings had been grown and the great accumulation of aluminum on the roots of the seedlings is also a matter of interest, although it has no immediate bearing on the subject under consideration. It is also evident from the foregoing that there is a decided accumulation of translocated mineral matter in the leaves of the seedling, a fact that is in harmony with the rapid growth of the leaves as compared with that of the other parts of the seedling.

EXPERIMENTS WITH POTATOES

In the experiment with the potato tuber we have allowed potatoes to sprout in a dark closet, after they had been thoroughly cleaned. When the tubers began to soften they were removed from the dark closet and the sprouts (1) cut off. The potato was then carefully pared and the skin (2) and the starchy tissue (3) were carefully dried and ashed and the quantities of calcium, magnesium, phosphorus, potassium, and silicon were determined. The results are shown in Table V.

TABLE V.—*Analysis of separate parts of sprouted potatoes*

Part.	Part No.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried solids.	Phosphorus as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.	
		Gm.	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*
New sprouts.	1	21.3722	2.1185	9.91	0.2661	12.56	0.0190	0.90	0.0443	2.72	0.8558	40.40	0.0201	0.95
Skin	2	46.9359	3.8205	8.14	.2250	5.89	.0649	1.70	.0518	1.33	1.5408	40.33	.3228	8.45
Tubers (exhausted)	3	185.3766	8.1120	4.37	1.0058	12.40	.0608	.75	.1835	2.25	4.3415	53.52	.0486	.60
Total weight.	.....	253.6847	14.0510	.....	1.4969	.....	.1447	.....	.2796	.....	6.7381	.....	.3915	....

The relatively high percentage of ash in the sprout of the potato as compared with that contained in the exhausted tuber is a matter of interest. It will be seen, however, that considerable amounts of ash still remain in the exhausted tuber after the growth of the sprouts is complete, indicating the necessity of mineral matter for those changes occurring in the tuber during the act of sprouting. Table VI gives the percentage distribution of the several mineral constituents between the sprouts and exhausted tubers, including the skin.

TABLE VI.—*Percentage distribution of the mineral constituents of potatoes*

Part.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2).
Sprouts	17.77	13.12	15.84	12.68	5.13
Tubers (exhausted)	67.13	42.02	65.68	64.43	12.41

In Table VI it is observed that a large amount of the mineral material remains unused in the exhausted tuber of the potato and that approximately only 15 per cent of the different mineral constituents have migrated to the sprouts.

CONCLUSIONS

The most striking fact brought out thus far by these studies on the translocation of the mineral matter of the seed and tuber during the growth of the seedling is the retention of considerable amounts of the mineral matter, varying from 46.66 per cent in the garden bean and 38.66 per cent in corn to 50.33 per cent in the potato tuber in the cotyledons and tuber, respectively. As indicated in the foregoing experiments, this probably finds its explanation in the necessity for definite amounts of the various mineral constituents to promote the katabolic changes occurring in the cotyledon and tuber during sprouting. So far as could be ascertained, there were no very striking differences in the quantities of its several mineral constituents translocated and no marked selective influences shown by the roots, stem, and leaves of the growing seedling for any particular mineral reserve material contained in the seed or tuber. Up to the present time, great difficulty has been experienced in the selection of a suitable container in which to grow these seedlings. This has proved a serious obstacle to this work. It is hoped, however, that this difficulty may be finally overcome and better and more constant results obtained through the use of pure paraffin containers.

FATE AND EFFECT OF ARSENIC APPLIED AS A SPRAY FOR WEEDS

By W. T. McGeorge,
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INTRODUCTION

In certain districts of Hawaii during the rainy season cultivation is impracticable, because of its bad effect upon the texture of the soil. Yet at times this season is abnormally long and especially favorable to the growth of weeds. Weed control is therefore a very important problem for Hawaiian planters. In experiments at the Hawaii Experiment Station [1] it was found that the most economical means of weed control under such conditions lay in the use of chemical sprays. Careful comparative tests were made of such chemicals as sodium arsenite, ferrous sulphate, carbon bisulphid, etc. Of these, sodium arsenite proved by far the most effective and was recommended for use. Sodium arsenite sprays have now been used in Hawaii for weed eradication for about five years and have proved to be efficient and economical. Such sprays have not only been used to replace hand labor in the fields, but also as a means of ridding grass lands of undesirable plants.

In view of the possible injury to soils and crops as a result of the continued use of such sprays, the Hawaii Experiment Station undertook a study of the fate in the soil of the arsenic so applied and its influence upon plant growth and upon ammonification and nitrification.

EFFECT OF SODIUM ARSENITE ON PLANT GROWTH

Apparently there is little or no immediate danger to crops from the use of sodium arsenite as a spray. In fact, in experiments with millet, buckwheat, and cowpeas grown on three different types of Hawaiian soils it was found that small quantities of arsenic stimulate plant growth. However, analyses of the plants did show that the arsenic is assimilated and that when it is present in the tissues in sufficient concentration death of the plant results.

The most surprising feature of the investigation was the influence on the ammonifying and nitrifying bacteria. In one type of soil ammonification was stimulated even by such excessive amounts as 1 per cent of arsenic (As_2O_3) in the soil. The results as a whole indicate that no fear need be entertained regarding any detrimental influences toward the

[1] Wilcox, E. V. Killing weeds with arsenite of soda. Hawaii Agr. Exp. Sta. Press Bul. 30, 15 p. [1911.]
Krauss, F. G. Suppression of weeds among pineapples by arsenite of soda spray. Hawaii Agr. Exp. Sta. Press Bul. 48, 8 p., 2 fig. 1915.
McGeorge, W. T. The effect of arsenite of soda on the soil. Hawaii Agr. Exp. Sta. Press Bul. 50, 16 p., 3 fig. 1915.

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organisms upon which the plants rely for their available nitrogen, provided proper soil texture is maintained.

Furthermore, it was found that in time the arsenic practically loses its toxic influence toward plants. This was shown by the comparative growth of plants on soils treated at time of seeding and those seeded several months following the application of the arsenic to the soil. There are only two possible explanations of this condition: Either the arsenic reacts with certain of the soil constituents, resulting in a less toxic combination, or it is rapidly leached from the soil.

ABSORPTION OF ARSENIC BY THE SOIL

When a soluble salt is added to a soil, its ultimate disposition must depend upon certain chemical reactions and physical phenomena. In this case the possibilities involve (1) a combination with or replacement of salts already present, resulting in its absorption as a whole; or (2) a selective absorption involving the fixation of only one ion of the salt.

In order to determine the fate of arsenic and the effect of irrigation, a set of lysimeter experiments was inaugurated.

LYSIMETER EXPERIMENTS

Three types of soil were selected: (1) A ferruginous red clay, (2) a ferruginous brown clay, and (3) a highly organic silt. Twenty-five pounds of soil were placed in each of six lysimeters, two being filled with each type. To each soil were added 3 liters of a solution of sodium arsenite of the same strength as that used for spraying weeds. One series of three was allowed to stand for two months protected from rain. To the other three 1 liter of water was added every other day for several weeks, after which the soil was allowed to stand in the lysimeter until dry enough to sample.

The object of these experiments was to determine the rate of fixation, the depth to which the arsenic can penetrate, and the leaching effect of irrigation. At the expiration of the above time samples were taken at various depths in the lysimeters and the percentage of arsenic (As_2O_3) in the soil at each depth was determined. The results are given in Table I.

TABLE I.—*Effect of irrigation on arsenic in the soil, giving the percentage of arsenic at various depths*

Soil No. 1.			Soil No. 2.			Soil No. 3.		
Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.
Inches.	*Per cent.*	*Per cent.*	*Inches.*	*Per cent.*	*Per cent.*	*Inches.*	*Per cent.*	*Per cent.*
1 to 3	0.280	0.224	1 to 3	0.450	0.237	1 to 2	0.97	0.95
3 to 5	.198	.211	3 to 5	.170	.092	2 to 4	.50	.47
5 to 7	.171	.145	5 to 7	.118	.092	4 to 6	0	0
7 to 9	.184	.170	7 to 9	.013	.....	.....	.....	.....

The columns headed "Not irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were protected from rain and which received no irrigation. The columns headed "Irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were subjected to irrigation. A comparison of the two columns for each soil will show the strong fixing power of these soils for arsenic, the influence of different soil types upon the fixation, and the danger of its accumulation. Samples of soil No. 3 were taken at depths different from those of soils Nos. 1 and 2, as shown in Table I, because of the concentration of arsenic at the surface in the former.

In order to determine how nearly these results represent actual field conditions, samples of soil were obtained from a plantation at Nahiku, Maui, which was the first to adopt the use of sodium arsenite as a means of weed control. Weeds on this land have been sprayed for five years, at the rate of three applications per year, using 5 pounds of arsenic (As_2O_3) per acre for one application. During this time the soil has received no cultivation whatever and the rainfall averages about 200 inches per year. The soil is very porous and there is very little run-off water. Samples were taken at three depths: Every 4 inches of the first foot. The surface 4 inches contained 0.00924 per cent of arsenic (As_2O_3), and none was present below this depth. A determination made by boiling the soil with water showed an arsenic content of 0.00006 per cent, or 0.6 p. p. m., soluble in water. That the arsenic fixed by soils in the lysimeters was partly soluble in water indicates that the fixation is due in part to physical influences.

CHEMICAL REACTIONS INVOLVED IN THE FIXATION

The composition of the spray as prepared by recommended methods may be either a solution of the acid salt ($Na_2O.2As_2O_3.2H_2O$) or the neutral salt ($Na_2O.As_2O_3$), depending on the proportions of soda (either hydrate or bicarbonate) and arsenious acid used.

$$H_2O+2NaOH+2As_2O_3=Na_2O.2As_2O_3.2H_2O.$$
$$H_2O+2NaOH+As_2O_3=Na_2O.As_2O_3.2H_2O.$$

For the following experiments in studying the replacement phenomena, a solution of the neutral salt was used.

One liter of a 1 per cent solution of sodium arsenite was allowed to act upon 200 gm. of soil, with occasional shaking, for two weeks. Checks were also maintained with 200 gm. of soil and 1 liter of water. The arsenic extract was then separated from the soil and a partial analysis made to determine the elements with which the sodium arsenite is most active. The results are given in Table II, which shows the composition of a 1 per cent sodium-arsenite solution after contact with the soil, as compared with the solvent action of water. The percentage of humus

in the soil before and after treating with 1 per cent of sodium arsenite is also given.

TABLE II.—*Composition of the extracts (mgm. per liter)*

Constituent.	Soil No. 1.		Soil No. 2.		Soil No. 3.	
	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.
Fe_2O_3	Trace.	716	Trace.	121	Trace.	90
CaO	11.2	84	13.6	124	74.6	126
MgO	3.6	20	10.8	44	7.4	26
As_2O_3	.....	3,960	.....	6,000	.....	4,480
$Mg.As_2O_3$ fixed by 100 gm. soil	.....	2,640	.....	600	.....	2,120
Humus I[a], per cent	2.77		1.68		8.75	
Humus II[a], per cent	1.56		1.80		8.40	

[a] Humus I shows the percentage of the humus content of original soil; humus II, that of soil after treatment with the 1 per cent sodium-arsenite solution.

Table II shows a replacement of and a solvent action toward iron, calcium, magnesium, and humus, and suggests several theories as to the nature of the reaction. The soil absorbing the largest amount of arsenic lost through solution or replacement the most iron and humus. The soil absorbing the least arsenic lost the least iron and no humus. Apparently the absorption of arsenic by soil No. 3 is largely a mechanical fixation, as the data show a high absorption, but a low replacement.

In sodium arsenite we have the combination of a strong base with a weak acid. A well-known property of such salts is to react alkaline when dissolved in water. This is due to the faint dissociation of H_2O into $H+$ and $OH-$ ions. Here the chemical and physical phenomena involved in the fixation of sodium arsenite are directly or indirectly a result of hydrolysis. The latter term as used herewith is intended to convey the increased dissociation in a solution of sodium arsenite, which itself is only faintly dissociated. This results in an increase in the concentration of the hydroxyl ion and the formation of the highly dissociated electrolyte sodium hydrate, which in the soil would probably be rapidly converted to bicarbonate. In this form it would have a solvent action toward the iron and humus and more or less toward the magnesium and calcium through the formation of slightly soluble bicarbonates. Magnesium bicarbonate is very unstable as compared to calcium bicarbonate and, hence, is precipitated following the solvent action of the sodium bicarbonate. The calcium is more soluble even in the soils containing much higher amounts of magnesium. These reactions leave the arsenic free as the negative ion to combine with the dibasic and tribasic metals to form slightly soluble arsenites or arsenates, thereby fixing the arsenic in the soil.

The rate and extent of fixation of arsenic vary in different soil types, owing to the concentration and solubility of the basic constituents—i. e., dissociation was found to be more rapid in some soils than others. To illustrate, the soil absorbing the greatest amount of arsenic exhibited the strongest alkalinity and showed the greatest chemical activity. Furthermore, this same soil contained the least amount of the soluble bases, calcium, magnesium, and potassium, indicating that the chemical fixation is influenced by the pressure of soluble bases.

SUMMARY

It has been shown herein that soils possess strong fixing power for arsenic and that when a sodium-arsenite spray is used for destroying weeds the arsenic will ultimately be deposited in the surface soil, there to remain in spite of the leaching effect of rains or irrigation.

The chemical reactions involved in the fixation are a replacement or solution of iron, calcium, magnesium, and humus, owing in part to a hydrolysis of the sodium arsenite in solution, also a combination with the dibasic and tribasic elements to form the difficultly soluble arsenites or arsenates.

ANGULAR LEAF-SPOT OF CUCUMBERS

By ERWIN F. SMITH, *Pathologist in Charge*, and MARY KATHERINE BRYAN, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

INTRODUCTION

The angular leaf-spot of cucumbers (*Cucumis sativus*) has been known in the field for many years, but up to the present time no organism has been named as its cause, though it has been generally conceded to be of bacterial origin. The disease is characterized by the formation of numerous, often confluent, angular, dry, brown spots which by dropping out or tearing give the leaves a ragged appearance.

The literature on the subject, aside from mere notes on the occurrence of the disease scattered through pathological literature, consists of four papers by O. F. Burger, of Florida,[1] and a more recent Italian paper by Traverso.[2] Burger mentions the leaf-spot as preliminary to a more destructive fruit-rot, said to be due to the same organism. His description of the diseased leaves agrees with the appearance of leaves sent to the writers from Wisconsin, as well as with those obtained by them from other States, and with the leaf-spots which they obtained in Washington by pure-culture inoculations. A brief description of the causal organism is given in each of his papers, in one case with the group number according to the chart of the Society of American Bacteriologists. Burger's descriptions agree in the main except as to flagella and the diameter of his organism. In his earlier descriptions it is said to have polar flagella, but in the later ones it is reported to be peritrichiate. No name is given to the bacillus.

Traverso's paper is only a preliminary one, but it leaves no doubt as to the identity of the Italian and American disease. A motile, fluorescent, nonliquefying organism was isolated by him and inoculations were made with it, but no positive results were obtained (p. 459).

Who first reported this cucumber disease in the United States is uncertain; the senior writer has known it for 20 years, and several years ago (1904) plated out two yellow bacteria with which unsuccessful inoculations were made. Again, in 1907, at his suggestion, Mr. John R. Johnston, then of the Laboratory of Plant Pathology, made platings

[1] Burger, O. F. A new cucumber disease. *In* Fla. Agr. Exp. Sta. Rpt. [1911]/12, p. c–ci. 1913.
——— A bacterial rot of cucumbers. *In* Phytopathology, v. 3, no. 3, p. 169–170. 1913.
——— Bacterial rot of cucumbers. *In* Fla. Agr. Exp. Sta. Rpt. [1912]/13, p. xc–xciv, fig. 11–13. 1914.
——— Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121, p. 97–109, fig. 37–42. 1914.

[2] Traverso, G. B. Sulla bacteriosi del cetriolo in Italia. Nota preliminare. Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., s. 5, v. 24, sem. 1, fasc. 5, p. 456–460. Apr. 5, 1915.

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and isolated a yellow schizomycete with which unsuccessful inoculations were made on cucumbers in the Department greenhouses.

ISOLATION AND IDENTIFICATION OF ORGANISM

Specimens were sent to the Laboratory of Plant Pathology in August and September, 1914, from New York and Wisconsin. No complaint was made by the sender of any association with fruit-rot, either on his own initiative or when questioned.

The interior of the spots was found to be swarming with bacteria which on floating out on the slide showed active motility. Plates were poured from such spots and a white, motile, rod-shaped organism was isolated. Spray inoculations with subcultures from three colonies on these plates gave typical infections on young cucumber leaves, from which the organism was reisolated. Colonies (subcultures) from this reisolation were then used for spray inoculations, and again the typical disease was produced with great virulence.

In August, 1915, specimens were received from several localities in Wisconsin, Indiana, and New York and from Ontario, Canada. In each case the same organism was isolated in pure cultures and used to produce typical infections on cucumber leaves in the hothouse.

The organism causing the angular leaf-spot of cucumbers appears to be an undescribed form for which the specific name *lachrymans* is suggested on account of the tearlike drops of exudate from the spots in early stages of the disease. Its brief Latin diagnosis is as follows:

Bacterium lachrymans, sp. nov.

Baculis cylindricis apicibus rotundatis, solitariis, saepe binis; baculis unis 0.8×1–2μ; 1–5 flagellis polaribus mobilibus; aerobiis, asporis.

Habitat in foliis vivis Cucumeris sativi in maculis angularibus. Liquefacit gelatinam lente. Colonae superficiales in agar-agar, rotundae, albae; colonae juvenes habientes centra non-translucida, et margines translucidas cum lineis multis radiantibus. Lac sterile alkalinum et translucidum fit; casein non segregatur. Nitrum non redigitur; culturae in mediis cum saccharo sacchari et saccharo uvae acidae fiunt. Gas non facitur. Methodo Grami non coloratur.

The organism which the writers isolated from the Wisconsin cucumber leaves and have here designated "*Bacterium lachrymans*, n. sp." differed culturally is so many important respects from Burger's organism that all our cultural experiments were repeated. These repetitions, however, confirmed the differences, which are given in Table I.

While it is not doubted that Burger had this disease under observation, it is believed that the organism described by him is not its cause, but is rather the cause of a rapid soft-rot of the fruit. His organism, however, may be a wound parasite following injuries due to the organism here described.

TABLE I.—*Differences between Bacterium lachrymans and Burger's cucumber organism*

Bacterium lachrymans.	Burger's organism.
1. Polar flagellate	Peritrichiate flagellate.
2. Liquefies gelatin	Does not liquefy gelatin.
3. Clears milk without coagulation	Coagulates milk.
4. Strict aerobe (does not grow in closed end of fermentation tubes).	Facultative anaerobe (grows in closed end of fermentation tubes).
5. Forms acid from saccharose in fermentation tubes.	Does not form acid from saccharose in fermentation tubes.
6. Forms acid from dextrose in fermentation tubes.	Does not form acid from dextrose in fermentation tubes.
7. Not villous along line of stab in either agar or gelatin.	Villous along line of stab in both gelatin and agar.
8. Does not become yellow with age on sugar agars.	Becomes yellow with age on sugar agars.
9. Moderate indol formation	No indol formation.
10. Agar-plate surface colonies show many fine radiating lines.	Agar-plate colonies homogeneous in structure.
11. Does not cause soft-rot of cucumber fruits.	Causes a soft-rot of the fruit.
12. Surface colonies on agar plates are always round.	Agar colonies are round to ameboid.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Mr. Frederick V. Rand, of this laboratory, by whom these specimens were collected, reported the disease in 1915, from the following localities:

MICHIGAN: Big Rapids, Muskegon, Grand Haven, Holland, Grand Rapids, and Hudsonville.

INDIANA: Plymouth, Monterey, Tyner, and Donaldson.

WISCONSIN: Racine, Portage, Ripon, Princeton, and Milwaukee.

NEW YORK: Constable, Malone, North Lawrence, and Long Island.

CANADA: Provinces of Ontario and Quebec.

In regard to the amount of injury caused by this disease, Mr. Rand says:

In most cases I found the angular leaf-spot causing a rather minor injury, but in an occasional field I found all the leaves back of the tips of the vines very badly shot-holed and presenting an exceedingly ragged appearance, such that serious injury to the crop must inevitaby result. Last year this disease had done more damage than any other in the vicinity of Ripon, Wis.

This disease has also been reported recently from Maryland and several other Southern States.

Earlier the senior writer received specimens from Michigan, Wisconsin, Indiana, Connecticut, and the District of Columbia.

INOCULATION EXPERIMENTS

On October 26, 1914, young cucumber plants were sprayed in cages in the hothouse with water suspensions from young agar slants made from three colonies on the plates poured from diseased leaves. The plants were kept moist in the cages for 30 hours, then removed to the bench.

Five days later, water-soaked spots appeared on the leaves, and by November 3 there were typical browned spots on plants inoculated with each of the three colonies. These spots swarmed with bacteria. Poured plates on agar gave pure cultures of the same white organism. No further inoculations were made until April 30, 1915, when sprayings were again made in cages as before, using subcultures of colony No. 1, plated from a spot produced by the inoculations of October 26. The plants used in this case were of a common field variety and rather stunted but with sound leaves. Three days after the first spraying water-soaked spots appeared on the lower surface of the leaves, and by May 6 these had enlarged into the typical angular, dry, brown spots.

Another experiment on May 6, 1915, using perfectly healthy, free-growing Arlington white spine cucumber plants and subcultures from the same colony (No. 1) gave striking results. Several leaves showed tiny water-soaked areas on the second day, and all the leaves were typically and badly spotted by the sixth or seventh day. In this stage the spots were one-fourth to three-fourths of an inch in diameter, angular, following the larger veins, and water-soaked (translucent), not dry. In the early morning drops of moisture (exudate) swarming with bacteria were found hanging on the lower surface of such spots (Pl. XLV, fig. 1). Pure cultures of the causal organism were obtained by plating from one of these drops. On the following day, or even later on the same day, white films (bacterial crusts) replaced the drops (Pl. XLIII, fig. 1). The appearance of infected leaves at the end of 12 to 14 days, when the diseased areas have become dry and begin to drop out, is shown in Plate XLIII, figure 2.

As the young unsprayed leaves developed on these plants, they became naturally infected; and in three cases the stems and petioles of this young growth also became water-soaked, exuded drops of fluid (Pl. XLIV, X, X), and finally broke or bent over (Pl. XLV, fig. 2), ending the growth of the plant. The cracking open of stems in this stage of the disease is shown at X in Plate XLV, figure 2, and in detail in Plate XLV, figure 3.

On the green fruits up to the end of August, 1915, the writers were able, with one exception, to obtain within a week or 10 days (shipping time) only a local infection and a bacterial exudate such as that shown in Plate XLVI, figure 1—no general soft-rot. Even when the fruit (Pl. XLVI, fig. 1) was kept for another week at high temperatures (28° to 32° C.), it did not rot (Pl. XLVI, fig. 2). Altogether 15 such fruits were inoculated with virulent cultures, some on the vines and others in damp chambers.

Soft-rot occurred twice in young fruits (two-thirds grown) when placed in damp chambers after inoculation. In the first case (the exception referred to above), plates were poured from the soft interior of the one fruit thus affected. As only spreading fimbriate colonies were obtained, the soft-rot was attributed to an intruder, and no further studies were

made. Some months later (September, 1915) in a similar experiment two out of four inoculated fruits became soft-rotted. These fruits were from the market. All four showed the local gumming at the point of inoculation (needle pricks) after five days, while check pricks gave no gumming. Two days later two fruits began to soften, and the next day the whole interior was swarming with bacteria. Plates were poured from the interior of one of these fruits under sterile conditions, and again only spreading fimbriate colonies were obtained. Smears from these colonies stained by Van Ermengem's flagella stain gave rods with as many as 8 or 10 peritrichiate flagella. This organism grew well in the depths of agar stabs and curdled milk with reddening of litmus in milk. The other two inoculated fruits remained sound and after two weeks when cut open showed only a very local infection not extending much beyond the needle pricks in any direction.

Since the organism causing the leaf-spot is polar flagellate and aerobic, does not develop a fimbriate growth on agar, and does not curdle milk or redden litmus in milk, it is evident that this soft-rot was due to an intruder, which may have come from the surface of the fruits, since they were not sterilized, but only washed.

When these fruits became soft-rotted, the suspicion arose that possibly the softening and cracking of the stems and petioles (Pl. XLV, fig. 2) might also have been due to some unsuspected soft-rot organism. The inoculation experiments with *Bact. lachrymans* were therefore repeated on stems and petioles of free-growing cucumbers with the same result as before—i. e., softening and cracking of the younger stems and petioles. From one of these stems platings were made and *Bact. lachrymans* obtained in pure culture. At the same time several control inoculations were made on stems and petioles, using a subculture of the fimbriate, peritrichiate, soft-rot organism plated from one of the softened cucumbers above mentioned, but no rot occurred (four weeks). This organism, however, soft-rotted green cucumber fruits when inoculated by needle pricks.

Last of all, following the discovery of Traverso's paper, another set of inoculations was made on cucumber fruits. Six marketable green hothouse fruits were selected and inoculated with *Bact. lachrymans*. At the end of 10 days in culture dishes at temperatures varying from 24° to 30° C. all showed local gumming and infection about the needle wounds, but none of them developed any soft-rot (Pl. XLVI, fig. 3).

HISTOLOGY OF DISEASED LEAVES

Pieces of a leaf that showed spotting were fixed on the second day, embedded, sectioned, and stained. Stomatal infections were very numerous (Pl. XLVII, fig. 1). The bacteria gorged the opening of the stoma in some cases, as well as the cavity beneath it. Even at this early date the bacteria had spread in great numbers for some distance from the stoma, crowding apart or crushing the cells of the parenchyma and causing a slight swelling on the leaf (Pl. XLVII, fig. 2).

MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM LACHRYMANS

MORPHOLOGICAL CHARACTERS

As it occurs in the plant and also on media the organism causing the disease is a short rod with rounded ends, single or in pairs (Pl. XLVIII, fig. 2 and 3), 0.8μ wide by 1 to 2μ long. On culture media it occurs singly or in pairs with a very decided constriction, and occasionally (in salted bouillons) in chains of as many as 12 or more individuals (Pl. XLVIII, fig. 1). No spores have been seen. Capsules are formed on agar (Pl. XLVIII, fig. 2), and in milk (Ribbert's stain). It is motile by means of 1 to 5 polar flagella (Pl. XLVIII, fig. 3). It is Gram-negative and is not acid-fast.

EFFECT OF DESICCATION

When drops from 24-hour peptone bouillon were placed on sterile covers in sterile Petri dishes and kept in the dark at room temperature, the organism was not killed by 21 days' drying, but it gave no growth when covers were dropped into suitable bouillon after 6 weeks' drying.

TEMPERATURE RELATIONS

The best growth was obtained at 25° to 27° C. There was no growth at 36°, though bouillon was weakly clouded at 35° C. Slow growth occurred at 1° in bouillon cultures (two weeks' time).

SENSITIVENESS TO SUNLIGHT

Agar plates, thin-sown, from an 8-day bouillon culture were exposed, bottom up on ice, to sunlight in June for 5, 10, and 15 minutes, one-half of each plate being protected from the light by several thicknesses of black paper. After five days' incubation numerous colonies appeared, and no difference was observed between the insolated and covered side on any of the six plates (but the colonies were not counted). Another test was made in September, 1915, with the following results:

The fluid used for inoculation consisted of one 3-mm. loop from a 24-hour bouillon culture into 10 c. c. of bouillon. Five plates were inoculated, each with one 2-mm. loop from this suspension. Five other plates were inoculated, each with one needle from this suspension. One plate from each lot was then half covered and exposed bottom up on ice for 5, 15, 30, 45, and 60 minutes, respectively. Result: All were killed by 45 and 60 minutes' exposure; three-fourths were killed by 30 minutes' exposure; one-third were killed by 15 minutes' exposure; and one-fourth were killed by 5 minutes' exposure.

When these results were obtained with the 24-hour bouillon, the experiment with the 8-day bouillon was repeated. Four agar plates were poured, one-half of each being exposed bottom up on ice, two for 15 minutes and two for 30 minutes, the sky being clear and the sun bright (October 12).

There was a marked reduction of colonies on the plates exposed for 15 minutes (estimated, 70 per cent), and almost complete absence of colonies on those exposed for 30 minutes (estimated, 95 per cent destroyed). The contradictory earlier result must therefore be attributed to a feebly actinic condition of the sky not visible to the naked eye.

SENSITIVENESS TO FREEZING

The organism is quite sensitive to freezing. A transfer was made to beef bouillon from a 5-day-old bouillon culture, shaken well and allowed to stand for five minutes. Plates were then poured with measured loops from this culture. The tube was then buried in salt and pounded ice, frozen solid and kept frozen for 15 minutes, after which it was thawed in cool water (five minutes required), shaken thoroughly, and used for a second set of plates, the loops being measured exactly as before. Two days after pouring the colonies were counted. There were one-ninth as many colonies after freezing as before freezing (Pl. XLVII, fig. 3). A longer incubation (five days) did not increase the number of colonies on the plates.

Thinking that five minutes might not have been long enough to obtain a uniform diffusion of the bacteria in the fluid, the experiment was repeated, allowing the tube to stand an hour with shaking before the plates were poured. The result was practically the same, nine-tenths of the bacteria being destroyed by the short freezing, the count being made on the fifth day.

CULTURAL CHARACTERS

AGAR-POURED PLATES.—On +15 peptone-beef agar at 23° C. surface colonies 2 days old are 1.5 to 2 mm. in diameter, round, smooth, shining, slightly convex, finely granular (under the compound microscope), with an opaque white center and a thin, transparent, entire margin. When 3 to 4 days old at 23° C. the largest measure 4 to 7 mm. in diameter and the white opaque center spreads in radiating lines into the thin margin (Pl. XLIX, fig. 1). At higher temperatures (27° to 30° C.) they reach this size in two to three days. Buried colonies are lenticular. Later (when 4 to 5 days old) the surface colonies lose their dense white center and dry down very thin and transparent and then show little or no trace of the radiating lines.

AGAR STABS.—Stabs in +15 peptone-beef agar when 2 days old at 23° C. show a raised, smooth, shining, white, transparent, surface growth 8 mm. in diameter. Growth is visible only along the upper one-third of the stab. This is granular, not villous.

Old cultures have a thin white growth completely covering the surface, and the agar is then frequently pale green, fluorescent.

AGAR SLANTS.—On slant agar, stroke cultures make a moderate, thin, white, transparent, smooth, shining growth, denser in the center. There is considerable white sediment in the V.

GELATIN PLATES.—Surface colonies on gelatin plates show a peculiar margin, best seen under low magnifications, with oblique light (Pl. XLIX, fig. 2). Liquefaction is slow (18° to 20° C.), and when the layer of gelatin is thin (10 c. c. to a plate) does not take place, as the medium soon becomes too dry for growth. On plates containing 20 c. c. of gelatin liquefaction began on the twelfth day and on the sixteenth day was complete, the colonies floating intact in the liquid gelatin.

GELATIN STABS.—At 15° to 18° C. in +10 peptone gelatin the surface growth after seven days is about 6 mm. in diameter, with a pit of liquefaction 2 mm. wide and 2 mm. deep. Stab growth is granular, not villous, fading out downward. As liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-form (Pl. XLIX, fig. 3). Liquefaction progresses rather slowly but is complete within three to four weeks at the specified temperatures.

BEEF BOUILLON.—In +15 peptone-beef bouillon uniform clouding occurs within 24 hours. This clouding is weak to moderate, never strong. On the second day a membranous pellicle is formed, which fragments and falls readily on shaking. It is made up of a homogeneous mass of bacteria—i. e., free from pseudozoogloeæ but containing a few short chains (10 or 12 individuals). Old cultures (4 to 6 weeks old) are often decidedly green fluorescent. The white precipitate breaks up readily on shaking and contains many small crystals.

POTATO CYLINDERS.—When inoculated from agar cultures growth on steamed potato cylinders in two days is moderate, spreading, creamy white, shining, and slimy. The part of the potato out of the water becomes slightly browned. Growth on potato soon ceases. After 10 days the color of the potato is completely changed, becoming a pale brownish hue, and the growth takes on a similar color (very pale brownish). Tested with alcohol iodin for starch, such cultures give a heavy dark-purple reaction, showing that there has been only a partial digestion of the starch (formation of amylodextrin). The cylinders are not softened.

MILK.—Inoculated milk clears slowly and without coagulation. Clearing begins within a week, and after two weeks tubes of it are translucent so that the outlines of a pencil back of the milk may be seen through it clearly. Cultures 1 month old are still clear but are then tawny olive,[1] with a darker rim where the milk has dried down.

LITMUS MILK.—Lavender-colored litmus milk begins to blue from the top downward on the second day and is completely blued by the third day, without a sign of coagulation or clearing. A decided creamy-white pellicle is formed.

After 10 days clearing begins and is complete in 20 days. Later the blue color bleaches out (reduction phenomena), beginning at the bottom, leaving the whole fluid a clear (translucent) brown. At no time is there any reddening of the litmus or any coagulation of the milk; nor are any crystals formed in it.

FERMENTATION TUBES.—The tests in fermentation tubes were made in water containing 2 per cent of Witte's peptone, to which was added 2 per cent of the carbon compound to be tested—namely, saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open end of each on the second day, heaviest in the tubes containing saccharose and dextrose, but the closed end in every case remained clear, with a distinct line across the inner part of the U. When 5 days old they were tested with neutral litmus paper. Saccharose and dextrose gave a decidedly acid reaction, while all the others were neutral. When 20 days old the saccharose and dextrose were still acid and the others weakly alkaline. No gas was formed and no growth occurred in the closed end of any.

No gas was formed in fermentation tubes containing sterile milk; nor was there any separation of the curd. The milk in the open end cleared gradually, while that in the closed end remained unchanged. The litmus reaction was alkaline in the open end.

Nitrate bouillon in fermentation tubes gave a good clouding in the open end, none in the closed end, no gas, and no nitrate reduction. A decided alkaline reaction was obtained with neutral litmus paper.

TOLERATION OF SODIUM CHLORID.—Neutral peptone-beef bouillons containing 2, 5, 6, and 7 per cent of chemically pure sodium chlorid, respectively, were inoculated from young bouillon cultures. Growth was retarded by 2 per cent of sodium chlorid

[1] Ridgway, Robert. A nomenclature of colors . . . 129 p., 17 pl. (partly col.). Boston, 1886.

and inhibited by all the other strengths. The experiment was repeated using 2, 3, and 4 per cent of sodium chlorid. Again, the 2 per cent retarded growth (clouding on the fourth day). Checks clouded after 24 hours. Growth appeared in the 3 per cent after 12 days, but there was no growth in the 4 per cent even at the end of four weeks. In both 2 per cent and 3 per cent the growth was scanty and flocculent, composed largely of chains (Pl. XLVIII, fig. 1), especially in the 3 per cent solution.

TOLERATION OF ACIDS.—Neutral bouillon containing 0.1, 0.2, and 0.3 per cent, respectively, of malic acid, tartaric acid, and citric acid was used. After three days the 0.1 per cent cultures of all three acids were well clouded; the 0.2 per cent malic and tartaric acids were all moderately clouded, while the 0.2 per cent citric acid showed no growth. None of the 0.3 per cent cultures were clouded. After three weeks the 0.2 per cent citric acid was well clouded, but in no case did the 0.3 per cent cultures show any growth. The cultures were watched for five weeks.

TOLERATION OF ALKALI.—The organism is quite sensitive to alkali. Peptonized beef bouillons titrating, according to Fuller's scale, +25, +20, +10, +5, 0, −5, −20, and −30, were inoculated from a 4-day bouillon culture, using a carefully measured 3-mm. loop for each tube. After 24 hours all showed growth except the −20 and −30. Heaviest growth occurred in the +25, weakest growth in the −5, which was flocculent instead of clouded. Five days later the same relative growth was evident throughout the series, but the −5 had become clouded and the −20 weakly flocculent. The −30 remained clear. After two weeks there was moderate growth in the −20, but none in the −30. The alkali used was sodium hydrate.

USCHINSKY'S SOLUTION.—In Uschinsky's solution growth is heavy, with a heavy membranous pellicle which falls readily as a whole. Greening of the media begins at the top on the second or third day and proceeds rapidly downward until the whole is a decided pale apple green. The medium does not become viscid.

FERMI'S SOLUTION.—At the end of 10 days a fine green fluorescence like that in Uschinsky's solution is visible. No fluorescence appeared in tubes of Cohn's solution inoculated on the same date for comparison.

COHN'S SOLUTION.—There is good clouding, heaviest near the top, but without a pellicle. Numerous floating crystals occur and the white precipitate is dotted with crystals. No greening occurs.

SUGAR AGARS.—No yellowing occurred on any of the sugar agars used. Cultures were made on beef-peptone agars containing, respectively, 2 per cent of saccharose, maltose, and dextrose, and in sugar agar without beef—i. e., containing only peptone and saccharose. The cultures were watched for eight weeks, during which time they remained white.

DOLT'S SYNTHETIC AGAR.[1]—Growth is abundant, covering the surface on the third day with a thin pink layer. Reddening of the dark agar begins on the second or third day; and after 10 days the color is changed throughout, although the lower half has not lost completely its purplish hue.

BOUILLON OVER CHLOROFORM.—Growth is not retarded in unshaken tubes of peptone-beef bouillon to which 5 c. c. of chloroform have been added.

REDUCTION OF NITRATES.—Nitrates are not reduced. Five-day-old cultures in nitrate bouillon were tested by the addition to each of 1 c. c. of boiled starch water, 1. c. c. of potassium-iodid water, and 10 drops of sulphuric acid. There was no color reaction.

INDOL.—There is a weak indol production in 2 per cent peptone water and in peptonized Uschinsky's solution. Tests were made at the end of the fifth and tenth days by the addition of 1 c. c. of the standard sodium-nitrite solution and 10 drops of the sulphuric-acid water to each tube. No reaction appeared until the cultures were heated to 70° C., when a feeble but decided pink color appeared. The checks gave no pink reaction. A better reaction was obtained in peptone water containing 0.5 per cent of sodium chlorid (Dunham's solution)—about one-third that of *Bacillus coli*.

[1] Contains litmus, glycerin, milk sugar, and dibasic ammonium phosphate.

HYDROGEN SULPHID.—Strips of filter paper soaked in strong lead-acetate solution and dried were suspended over cultures in peptone-beef bouillon, milk, steamed potato, carrot, and turnip. No browning of the paper occurred within six weeks.

METHYLENE BLUE IN MILK.—Methylene blue is rapidly reduced. Cultures were made in milk containing 4 per cent of a 1 per cent solution of methylene blue. Bleaching begins on the second day and is complete or nearly so in six days, except for a pale-blue surface layer 2 to 4 mm. deep and a deep-blue rim and pellicle. This pellicle, when examined under the microscope, is seen to be composed of masses of bacteria that have taken up the stain. When shaken repeatedly, these bleached cultures regain their blue color.[1]

BLOOD SERUM.—Stroke cultures on Loeffler's blood serum give a moderate, white, shining filiform growth 3 mm. wide. There is no liquefaction even after eight weeks and no color change in the substratum.

AEROBISM.—The organism appears to be strictly aerobic. It does not grow in the closed end of fermentation tubes with any carbon food tested. In agar stab cultures no growth occurs in the lower end of the stab. Cultures were also made by shaking an inoculated tube of melted agar, but no growth occurred more than 3 mm. below the surface. Stabs were made in agar, then 10 c. c. of melted agar poured on top. No growth occurred in the stab or at the junction point, but there was good growth on the exposed surface of the added agar.

LITMUS AGAR WITH SUGARS.—On litmus-lactose-agar stroke cultures there is moderate growth and no color change.

Stroke cultures on litmus-maltose agar give heavy growth, but do not alter the color.

On litmus-saccharose agar growth is heavy and the medium reddens, beginning at the thin upper end. The reddening begins on the second or third day and is complete on the fifteenth day.

Following the chart of the Society of American Bacteriologists, the group number is 211.23221*23.

EFFECT OF COPPER SULPHATE ON THE ORGANISM

Bouillon cultures 24 hours old were exposed to the action of chemically pure copper sulphate in the following manner. A dilution of copper sulphate (1 to 1,000) was made in a large Jena flask and allowed to stand overnight. After shaking thoroughly, further dilution was made again (in liter quantities) to 1 to 100,000 and 1 to 500,000. After these had been well shaken and had stood for an hour 10 c. c. of each were put into sterile test tubes and a loop of a well-clouded suspension from a 24-hour-old agar culture was added. Plates were poured after 5, 10, 20, and 30 minutes from each tube, using carefully measured loops. Checks were made by pouring plates with the same measured loops from a similar dilution in sterile water.

The plates were incubated at room temperature (27° to 30° C.). A colony count was made on the second day. Exposure to the 1 to 500,000 dilution gave no observed reduction of colonies, but the 1 to 100,000 destroyed nine-tenths of the organisms. The experiment was repeated with a strength of 1 to 50,000 of copper sulphate. All were killed at this exposure, while the check gave numerous colonies.

[1] The blue pigment is also absorbed by the bacteria from peptone water containing methylene blue.

*Nonchromogenic on most media, but green fluorescent in Uschinsky's solution, Fermi's solution, and old peptone-beef bouillon.

Some weeks later the experiment with copper sulphate was repeated. To liter quantities of distilled water in Jena flasks, chemically pure copper sulphate was added so as to obtain the following dilutions: 1 to 50,000; 1 to 100,000; and 1 to 500,000. Some hours after full solution, 10 c. c. of each dilution were pipetted into sterile test tubes and to each was added a 3-mm. loop from a heavily clouded water suspension made from a 24-hour agar slant culture. From each of these tubes three plates were then poured at the end of 5 minutes, and again three more at the end of 10 minutes. As a check, a 3-mm. loop of the cloudy bacterial suspension was added to 10 c. c. of distilled water and from this tube three plates were also poured. The agar for the first set of poured plates was seeded with a 3-mm. loop from the dilution tube, that for the second set with a 2-mm. loop, and that for the third set with a needle dipped one-half inch into the fluid. The results in colonies are given in Table II, the counts being made on the sixth day.

TABLE II.—*Effect of copper sulphate on Bacterium lachrymans*

Dilution used.	Number of colonies of *Bacterium lachrymans* developing in—						
	Checks.	1 to 50,000 copper sulphate.		1 to 100,000 copper sulphate.		1 to 500,000 copper sulphate.	
		5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.
Plate 1 (3-mm. loop)..	3,844	78	45	118	55	3,412	1,756
Plate 2 (2-mm. loop)..	2,296	27	16	29	44	2,400	916
Plate 3 (needle).....	22	0	0	0	0	12	5

SUMMARY

(1) The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States.

(2) It is characterized by angular brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish.

(3) Young stems and petioles may become soft-rotted or cracked open.

(4) A virulent outbreak often materially reduces the crop by destroying the needed active leaf surface.

(5) The spot is caused by *Bacterium lachrymans*, n. sp., which enters through stomata, no wounds being necessary. This organism is quite different from the one described by Burger [1] in his papers on cucumber rot. No direct connection has been found between the leaf-spot and the soft-rots of the fruit.

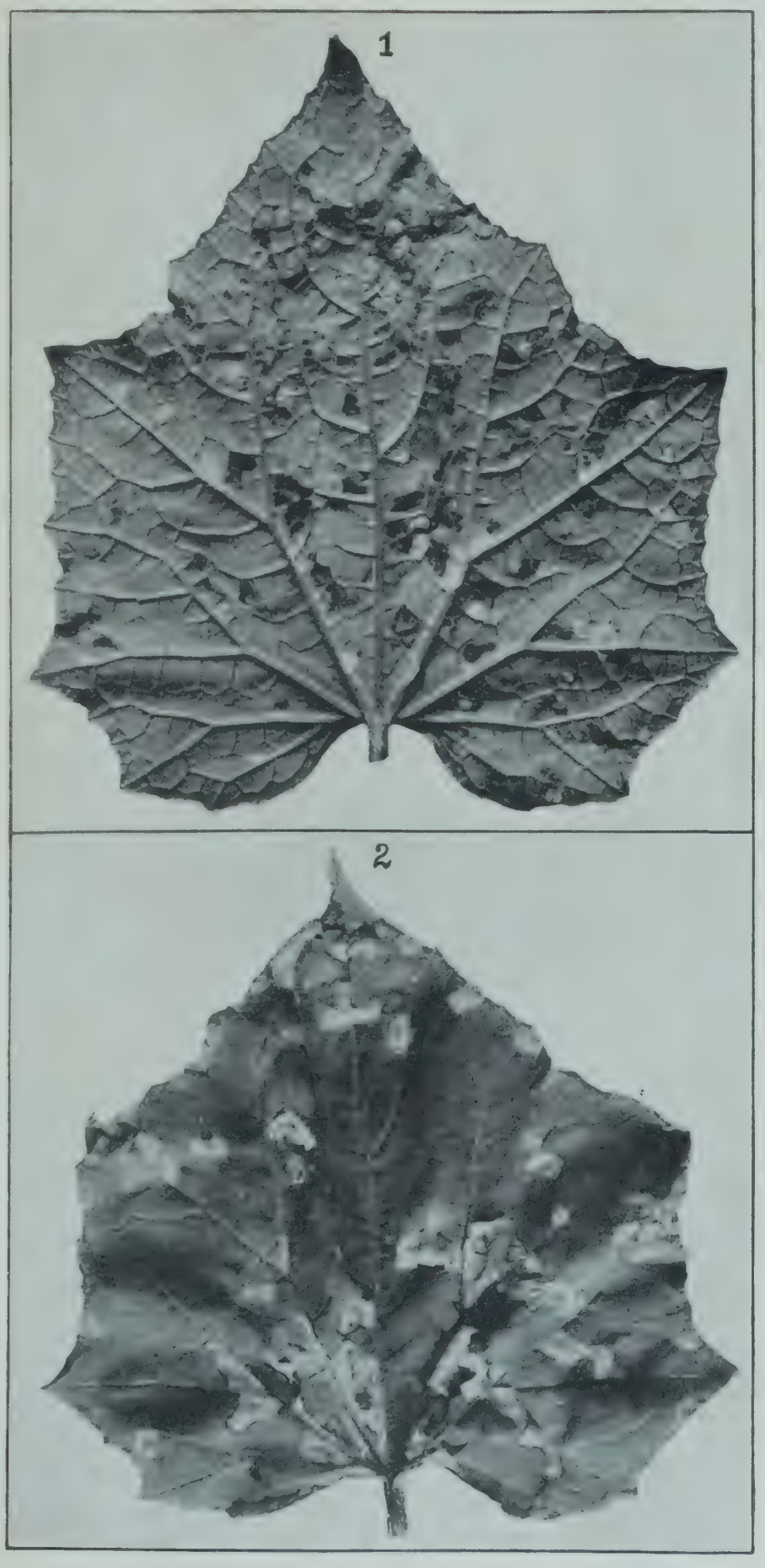
(6) Considering the results obtained in the laboratory with copper sulphate, it would seem that Bordeaux mixture properly applied is the remedy for this disease. Thorough field tests with it should at least be undertaken where the disease is troublesome.

[1] Burger, O. F. Op. cit.

PLATE XLIII

Fig. 1.—Cucumber leaf eight days after inoculation with *Bacterium lachrymans*. The bacterial exudate has now dried down into white crusts.

Fig. 2.—Cucumber leaf 12 days after spraying with *Bact. lachrymans*. Diseased tissue shriveled and spots falling out.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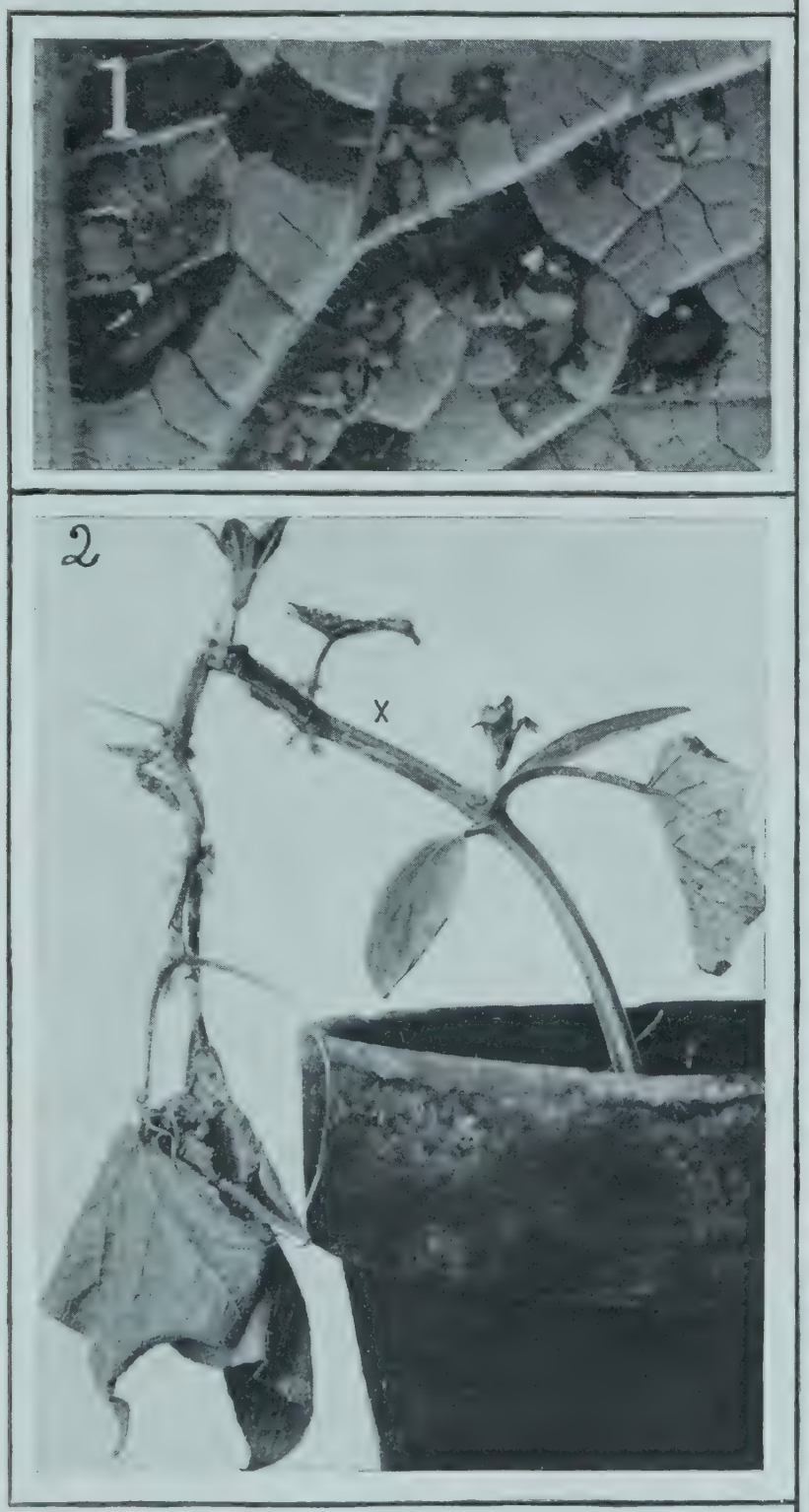
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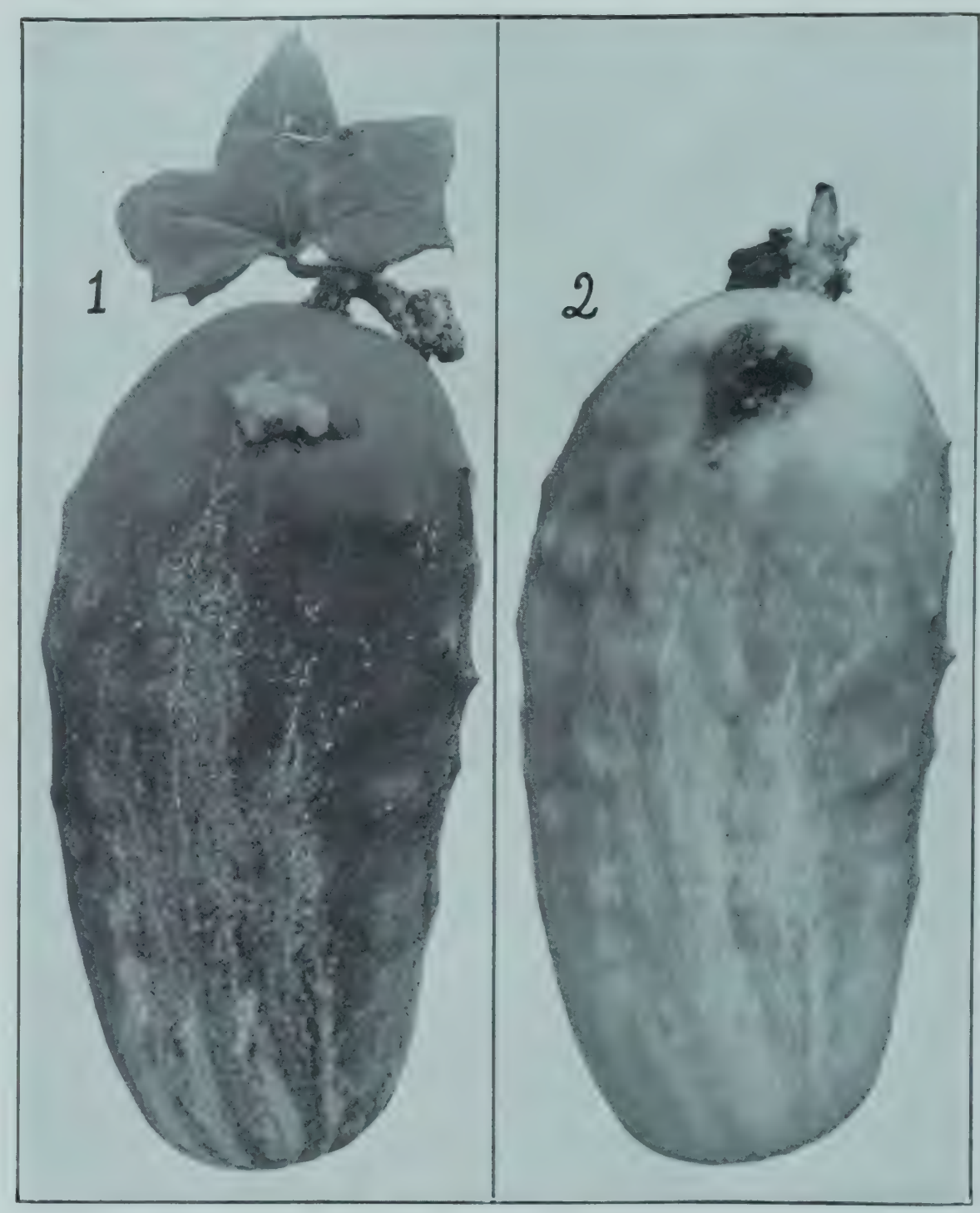
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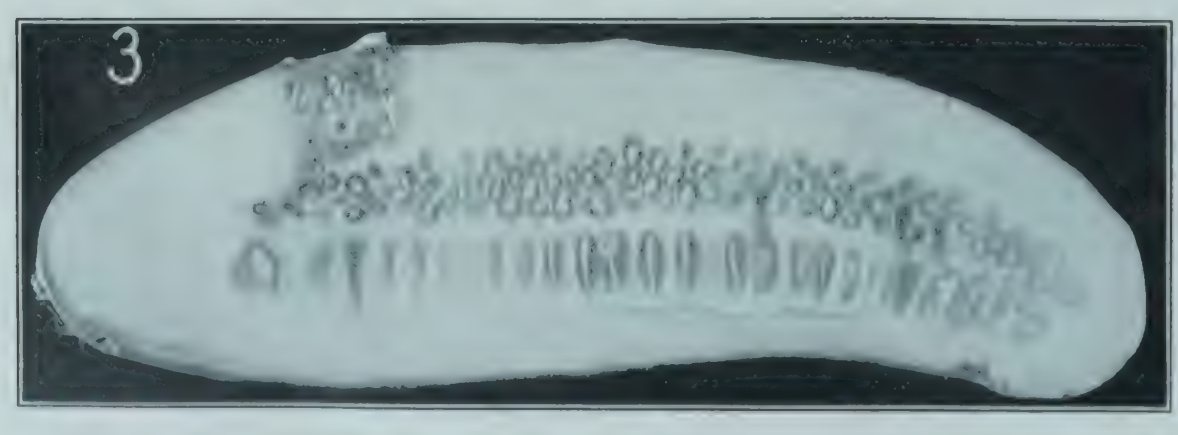
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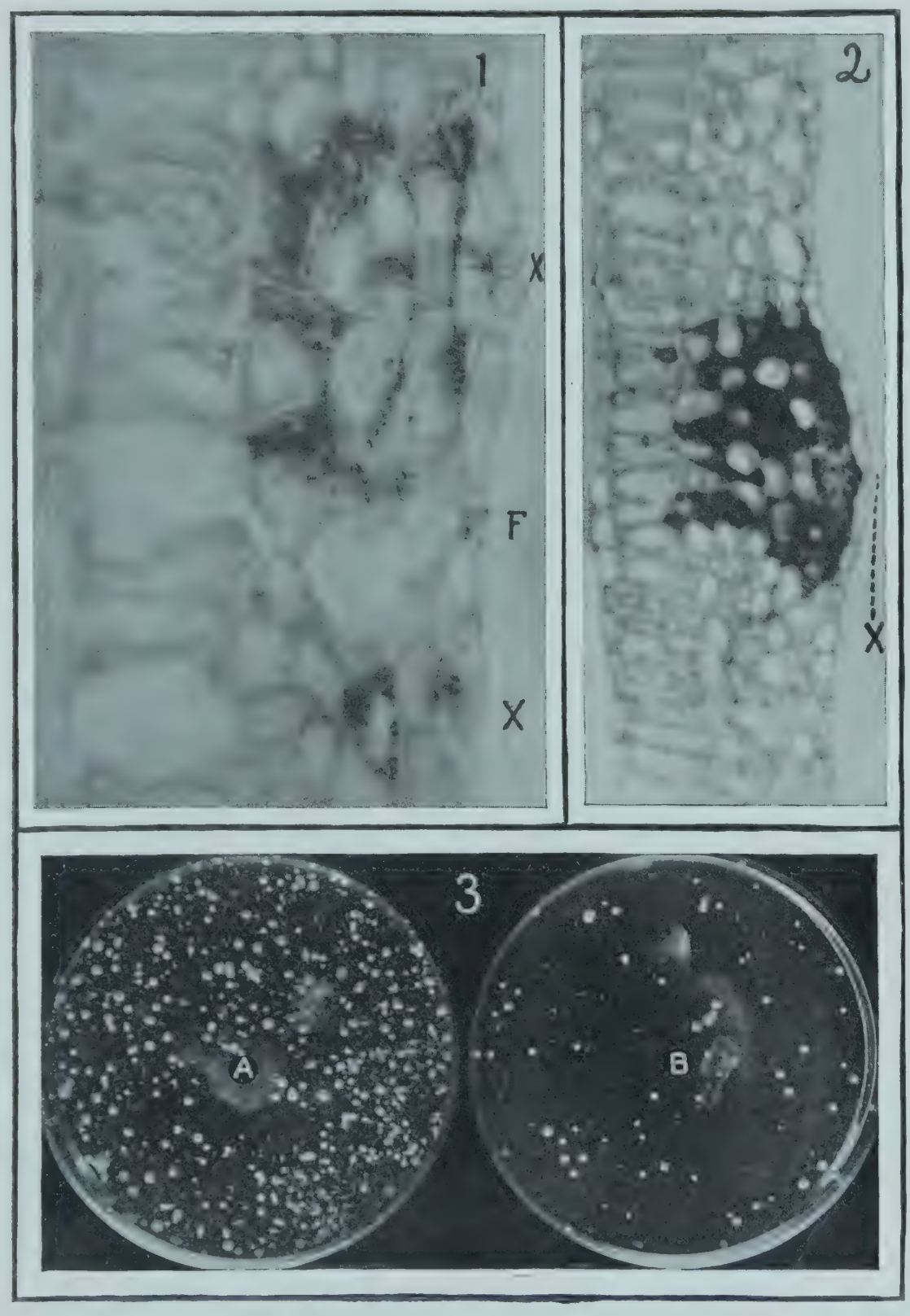
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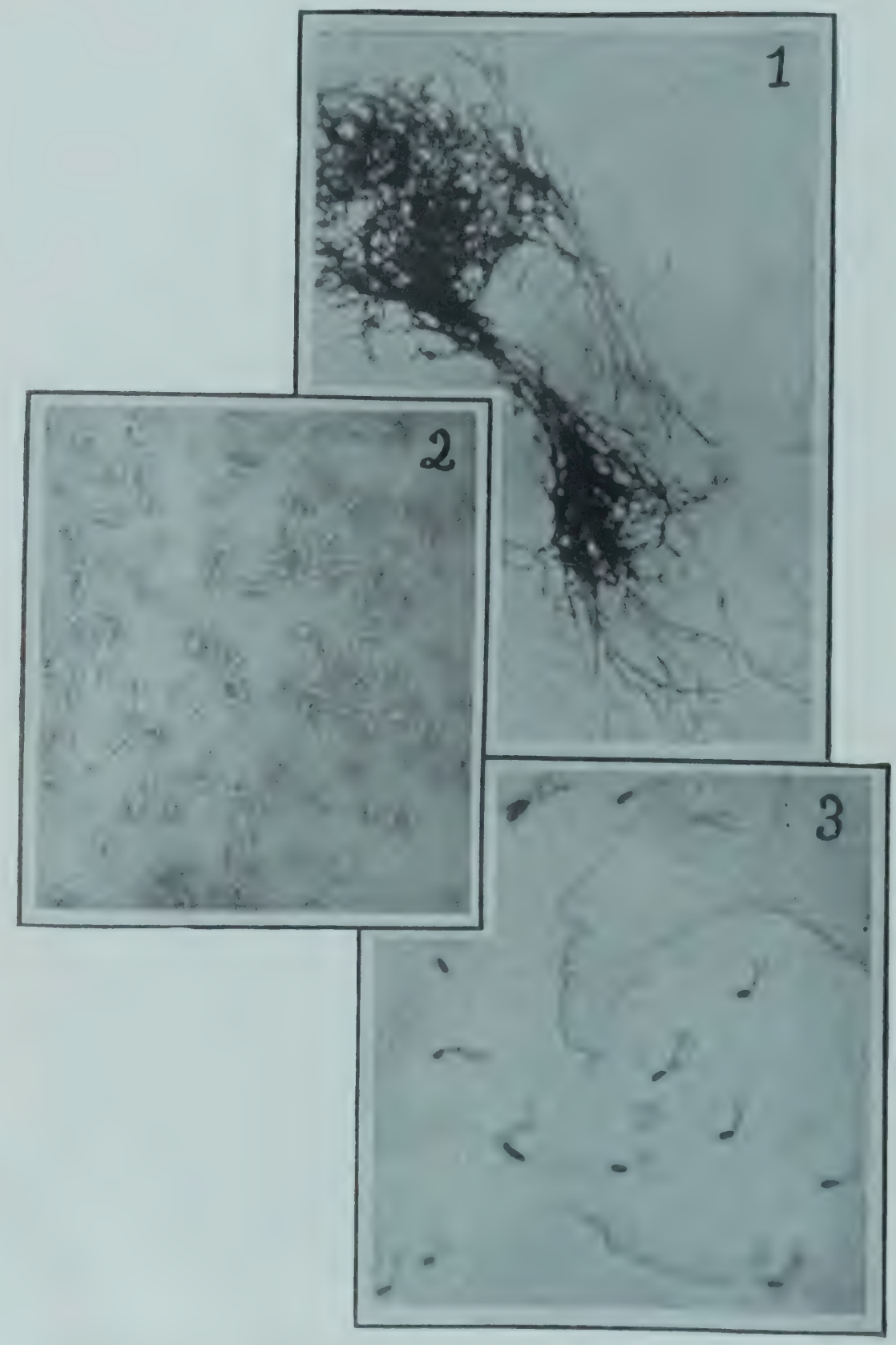
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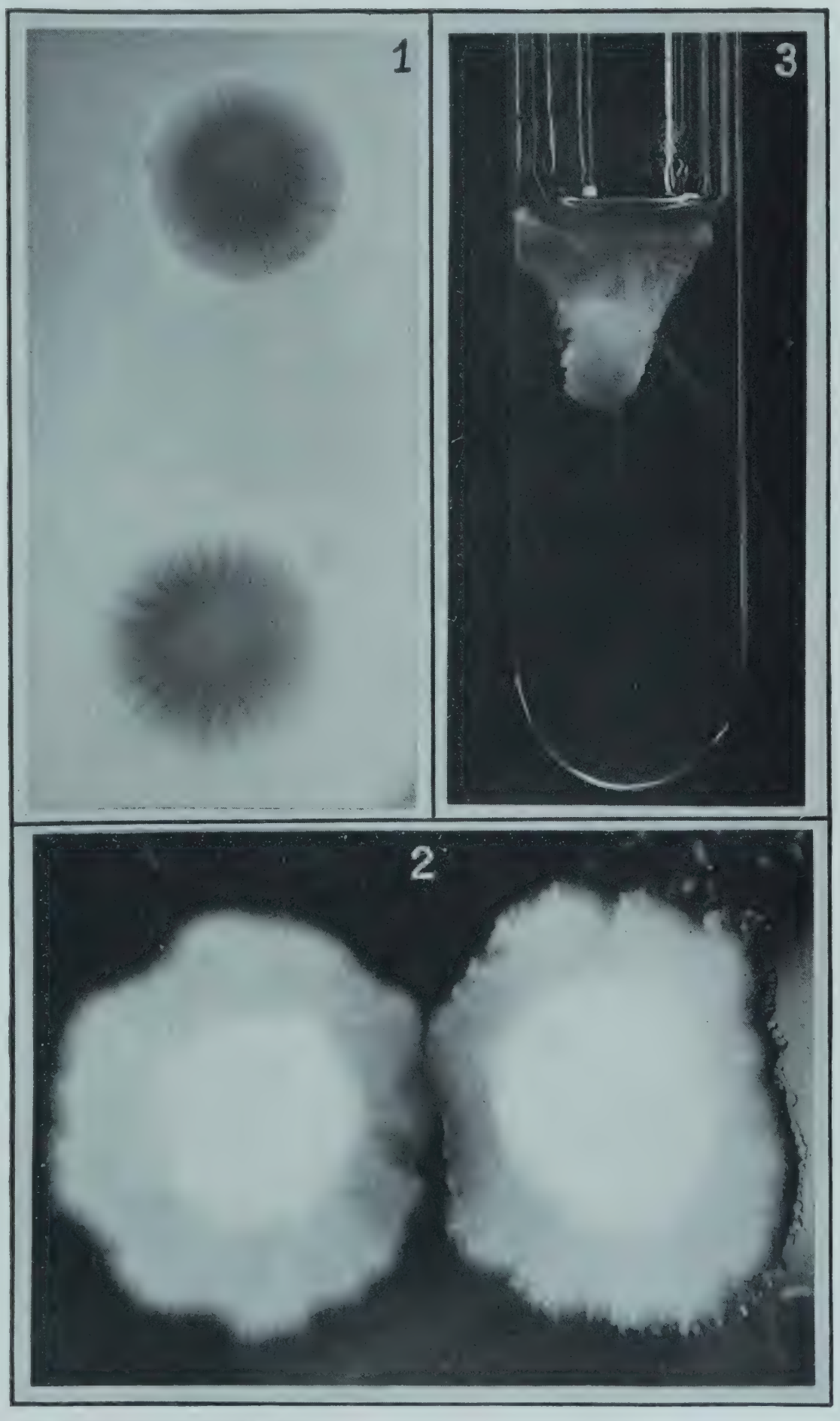
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ACTIVITY OF SOIL PROTOZOA[1]

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INTRODUCTION

The belief that soil protozoa are destructive to bacteria and, hence, are influencing factors in soil fertility is encouraging the more extended study of these organisms. It was shown elsewhere (5)[2] that the soil contains many cysts of protozoa which become active under favorable conditions. To serve as limiting factors in the soil, protozoa must be present in the active condition, for it is only as such that they can destroy bacteria and other micro-organisms; thus, the question at once presents itself, Are the protozoa active in the soil?

In 1909 Wolff (13) recorded investigations with soil protozoa undertaken for the purpose of ascertaining whether these organisms lead an active life in the soil and of discovering the factors which influence their activity. As to the presence of protozoa in the soil, Goodey (2), in 1911, concluded that they were not active in normal soils. A few years later, however, he (4) found that ciliated protozoa are in the encysted condition and concluded that the amebæ and flagellates were the limiting factors in the soil. Martin and Lewin (7) upon examining cucumber-sick soils found several different kinds of protozoa. The amebæ were probably the dominant type, and the flagellates were comparatively few. In 1911 Russell and Golding (9) noted that species of Vorticella, Putrina, Euglena, and other types present in ordinary soils were also found in sewage-sick soils. These organisms were more active in the sewage-sick soil than in ordinary field soil. In 1913 Russell and Petherbridge (11), in studying "sickness" in cucumber soil, found it to be full of organisms like myxomycetes, active amebæ, eelworms, and other lower animal forms.

Sherman (12, p. 630), who studied the presence of protozoa in several types of soil, summarizes his observations as follows:

> Certain forms of the soil protozoa are active under normal, and even sub-normal, conditions of moisture. The active protozoan inhabitants of most soils are probably restricted to flagellates. *Colpoda cucullus* is probably active whenever the moisture content is much above normal but does not appear to be so ordinarily.

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[1] Contribution from the Laboratories of Protozoology, Soil Bacteriology, and Soil Chemistry of the New Jersey Agricultural College and Experiment Station.

[2] Reference is made by number to "Literature cited," p. 488.

As to the activity of soil protozoa, Cunningham (1, p. 56) states:

> To the question as to whether the protozoa lead an active life in the soil, it has been shown that the action of heat combined with the dilution method does not give a definite answer. That question, however, is answered in the affirmative by the results of experiments which will now be discussed.

Martin and Lewin (8, p. 117) likewise in a recent article concluded that "it seems probable from the work that we have done up to the present that there are always some free living protozoa present in a trophic state in even relatively dry, poor soils."

In this study it is the purpose of the writer—

(1) To develop a method for studying protozoan activity in the soil.

(2) To ascertain whether the protozoa lead an active life in soils of different moisture content when the temperature is constant and when it is variable.

(3) To study the effect of moisture on the activity of the protozoa in the soil under constant and variable temperatures.

(4) To study the length of the period of excystment of soil protozoa.

METHOD FOR STUDYING PROTOZOAN ACTIVITY IN THE SOIL

In studying the activity of protozoa in the soil the first difficulty which is encountered is the lack of a suitable method by which the investigator can determine with certainty the extent to which these organisms are active in the soil. Several methods are recorded that have been used with more or less success. In 1911 Goodey (2) passed an electric current through the medium and found that the living protozoa traveled with the current to the cathode. The separation of active forms by centrifugation was attempted by Russell and Golding (10) in 1912. In 1913 Martin (6) discussed a simple method based on the mixing of a small quantity of soil with picric acid and then noting the organisms (bacteria, protozoa, and diatoms) which rose to the surface, when this mixture was placed in a wide dish and the soil stirred. Cunningham (1) employed the dilution method for examining and counting the protozoa in the soil. Martin and Lewin (8) discuss several methods which they have employed with more or less success. For the detection of living amebæ, an air-blast method which they have devised has proved to be the most successful.

It was suggested by Martin and Lewin (8, p. 110) that—

> Any method which depends upon the addition of water to the soil must admit of very rapid execution, otherwise there is danger of protective cysts present in the soil opening, and thus giving a false impression as to the constitution of the active fauna. This danger is probably a very real one in the case of small flagellates, and especially the resting forms of some green algæ, in the case of which a few minutes' immersion in water may make the difference between a resting and an active form.

In order to determine the presence of motile protozoa in the soil, the writer has found the direct method of examining the soil to which a little water has been added the most satisfactory.

Several drops of sterile tap water (15 pounds' pressure for 15 minutes) are placed on a clean slide; then by means of a stirring rod a small portion of soil is stirred in this water and spread out in a thin film, so that the observer can readily see between the soil particles. Examinations are then quickly made under the low power (16 mm. lens) of the microscope.[1] As soon as the soil touches the water, the time is recorded and the examination is continued for a period of not more than two minutes, in this way reducing the possibility of error which the observer might make on account of the rapid excystment of the protozoa, as was suggested above.

PROTOZOAN ACTIVITY IN SOILS OF DIFFERENT MOISTURE CONTENT AND UNDER CONSTANT AND VARIABLE TEMPERATURES

GREENHOUSE SOILS

The conclusions of other investigators as to the presence of protozoa in the active state in normal soils led the writer to examine greenhouse and field soils for the purpose of finding out, if possible, to what extent the protozoa were present in the active state in the different soils.

Twenty greenhouse soils of different composition and texture were examined, each for half an hour, a new sample being placed on the slide every two minutes. These samples were all taken at a depth of 1 inch from the surface. The examinations were all made in the greenhouse. The results are given in Table I.

From Table I it is seen that protozoa can and do exist in the active state in greenhouse soils. Their presence, however, is very limited, as they were found in but 6 out of the 20 soils examined. All the soils in which the protozoa were found were of open structure and their moisture content was much above their optimum. A compact shale soil with added manure and high moisture content did not show any living protozoa. Soils with a large proportion of organic matter and with a relatively low percentage of moisture did not seem to encourage the presence of active protozoa. From the data presented it would seem that the moisture content is the primary limiting factor, while the texture and content of organic matter are secondary.

[1] In studies previously recorded (1), all the examinations were made under the low power of the microscope, as it was not possible to distinguish between motile bacteria and what might be called "protozoa." In the studies referred to, no difficulty was encountered in seeing protozoa which were as small as species of Bodos or Monos; hence, the data collected in this study are based on the examinations made under the low power of the microscope.

TABLE I.—*Extent of protozoan activity in greenhouse soils*

Laboratory No.	Kind of soil.	Fertilizer treatment.	Termperature.	Moisture content.	Presence of protozoa.[a]
			°C.	*Per cent.*	
1	Clay loam.....	20 per cent of compost + minerals.	20. 8	26. 65	
2	Shale.....	20 per cent of compost.....	20. 9	34. 30	
3	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 0	26. 66	S.C.† A.†
4	Sandy.....	20 per cent of compost.....	21. 0	26. 84	
5	Clay loam.....	40 per cent of compost.....	24. 0	36. 27	
6	Shale.....	20 per cent of compost; 30 per cent of sand.	22. 7	25. 17	
7	Sandy loam....	No mixture.....	21. 6	22. 59	S.C.†
8	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 1	27. 57	
9	.....do.....	40 per cent of compost.....	21. 1	35. 75	
10	Sandy.....	20 per cent of compost.....	20. 8	26. 59	S.C.†
11	.....do.....	40 per cent of compost.....	23. 0	35. 35	S.C.† F.†
12	Sandy loam.....	.....do.....	22. 7	31. 28	
13	.....do.....	20 per cent of compost.....	22. 5	29. 10	S.C.†
14	Clay loam.....	20 per cent of compost + minerals.	24. 6	27. 90	S.C.†
15	Shale.....	20 per cent of compost + 10 per cent of sand.	21. 0	31. 75	
16	Clay loam.....	No mixture.....	19. 0	26. 21	
17	.....do.....	20 per cent of compost + minerals.	20. 3	31. 07	
18	Sandy loam....	20 per cent of compost.....	24. 0	25. 81	
19	Clay loam.....	20 per cent of compost; 20 per cent of sand.	24. 6	25. 09	
20	.....do.....	No mixture.....	18. 0	26. 60	

[a] S.C.=small ciliates; L.C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

FIELD SOILS

The extent of protozoan activity in field soils was studied in the same manner as the greenhouse soils. Samples of 14 field soils of different texture and tillage treatment were collected at a depth of 3 inches from the surface and brought to the laboratory in flasks. The temperature was in all cases noted. These were examined at once, each for half an hour, a new sample being placed on the slide every two minutes, as in the case of greenhouse soils. The moisture content was likewise determined. The soils were sampled and examined under normal conditions, again two days after a fall of 1.69 inches of rain, and a third time five days after 1.69 inches of rainfall. The second sampling was made at that period, since it allowed the organisms sufficient time to excyst, if possible, when the moisture content of the soil was increased. Likewise, the third examination was made five days after the heavy rainfall, for if the protozoa excysted and were washed to a lower level in the soil, this lapse of time allowed them to return to their normal level in the soil. Each soil was subjected to a half-hour's examination at every

sampling. In order to ascertain whether the soils contained cysts of protozoa which would become active when conditions became favorable after they had been examined, the soils collected at the third sampling were water-logged with sterile tap water and allowed to stand in the laboratory for 40 hours, when they were examined for motile protozoa. (See Table II.)

TABLE II.—*Extent of protozoan activity in field soils under different conditions of moisture*[a]

Laboratory No.	Kind of soil.	Soil treatment.	Normal moisture content.	Moisture content two days after heavy rain.	Moisture content five days after heavy rain.	Presence of active protozoa when soil samples were water-logged.[b]
			Per cent.	*Per cent.*	*Per cent.*	
1	Shale	Bare	25.07	22.01	21.73	S.C.††† F.†
2	Sandy loam	Orchard	13.73	18.66	14.18	S.C.†† F.††
3	Gravelly sandy loam.	Garden	9.62	12.40	8.60	S.C.† F.†
4	Clay loam	Orchard	15.14	19.18	12.72	S.C.† F.†
5	Gravelly clay	Meadow	15.67	20.10	15.21	S.C.††† L.C.† F.†
6	Clay loam	do	19.65	17.30	14.88	S.C.† L.C.† F.†
7	Silt loam	Wheat	11.22	15.24	9.38	S.C.†† F.†
8	do	Weeds	13.42	16.08	14.62	S.C.†† L.C.† F.†
9	Sandy	Corn	11.34	14.88	11.25	S.C.†† F.††
10	Gravelly silt loam.	Fallow	10.93	14.28	10.58	S.C.†† F.†
11	Shale	Bare	19.88	23.36	20.27	S.C.††† L.C.† F.†
12	Gravelly silt loam.	Wheat	9.60	15.51	8.97	S.C.† F.†
13	Silt loam	Corn	10.90	15.66	10.95	S.C.†† F.††
14	Sandy loam	Vetch and tomatoes.	6.74	12.18	8.52	Do.

[a] Under normal conditions and two and five days after a heavy rain no active protozoa were observed.
[b] S. C.=small ciliates; L. C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

The careful examination of the 14 soils in no case revealed any motile protozoa, indicating that under the normal and even somewhat abnormal conditions of moisture active protozoa did not seem to be present in the soils examined. Several samples of standing rain water were collected when the second and third samplings were made. Upon examination all of the samples of water showed the presence of many small ciliates and flagellates, which indicates that the protozoa are active in accumulated water. In all cases where the 14 soils were water-logged small ciliates and flagellates, and in some cases even large ciliates, were present in the active state. The data presented in Table II point to the fact that all ordinary soils contain cysts of protozoa, and in the 14 soils examined the active organisms were not observed until sufficient moisture was present. It would seem that if the protozoa did become active when the moisture content was higher than it was at the time of the first sampling after the heavy rain, they remained active but a very short period of time, as in no case were they

found in the living condition, while in soils of very open structure where little or no surplus water is available they would seldom, if ever, become active. This point requires further investigation.

The question at once arises, How are protozoan cysts transported to the different soils? This process is likely to be brought about by wind action, by flowing water, and by mechanical means in the case of cultivated soils. Likewise, if the protozoa do not exist in the active state in the soil, can they and do they multiply? Under certain abnormal conditions of moisture they will become active and remain active as long as there are sufficient moisture and food and the absence of toxic or decomposition products. During this period multiplication takes place. When the conditions become unfavorable, no doubt some die, while the greater number encyst until conditions again become favorable for them to become active.

EFFECT OF MOISTURE ON THE ACTIVITY OF PROTOZOA IN THE SOIL UNDER CONSTANT AND VARIABLE TEMPERATURES

Large samples of three soils which had previously been used by the writer (1) in his study of protozoa were collected. The first was a 20 per cent manure shale, greenhouse soil; the second, a clay loam orchard soil which had received no applications of manure for the last 20 years; and the third, a sandy loam field-plot soil that for a period of 20 years had been receiving annual applications of manure at the rate of 20 tons per acre. (Hereafter throughout this study the first soil will be designated as the "greenhouse soil," the second as the "orchard soil," and the third as the "field soil.") The soils were air-dried at laboratory temperature and then sieved through a 20-mesh sieve. The optimum moisture content of these soils was determined. Twenty 50-gm. portions of each soil were weighed into 4-ounce bottles. With each soil one series of five samples was left air-dried. To one series sufficient sterile tap water was added to make the moisture content half of the optimum. To another series enough water was added to increase the water content to the optimum. To a fourth series sterile tap water was added so that the resulting mixture would be equivalent to one and a half of the optimum. At one and one-half of the optimum the soils could take up all the moisture without any free water being present. The soils were well mixed with a stirring rod, so that the moisture content was homogeneous throughout. In order to prevent condensation on the sides of the bottles, they were left unplugged. The flasks containing four samples of each soil, representing four moisture contents, were incubated at 5° to 7° C., one series at 15° to 17°, one at 22° to 24°, one at 32° to 33°, and one at the outdoor temperature. The samples were weighed daily, and the slight amount of moisture lost by evaporation was replaced. Each sample of soil was then examined for active protozoa not fewer

than three times, a new sample being taken every two minutes; during the examinations the respective samples were kept at the different temperatures. Sterile tap water of the same temperature as that at which the respective soils were incubated was used in making the examinations. Each series of samples were kept screened from the light during the period of incubation. After examination the samples were again weighed to determine the quantity of soil used in examination. Daily examinations of each sample of each soil were made for a period of eight days. (See Table III.)

TABLE III.—*Presence of active protozoa in different soils, with varying amounts of moisture at different temperatures (constant and variable) for a period of eight days*

Laboratory No.	Kind of soil.	Moisture added to 50 gm. of soil.	Relative moisture.	Moisture content on the oven-dry basis.	Temperature of incubation.[a]	Presence of protozoa after inoculation (days).							
						1	2	3	4	5	6	7	8
		Gm.		*Per ct.*	°*C.*								
1211	Greenhouse soil.	0	Air-dry.....	0.69	15 to 17	....	....	.....	....	....	....	....	....
1212	.....do.....	4.96	½ optimum....	9.64	...do...	....	....	.....	....	....	....	....	....
1213	.....do.....	9.92	1 optimum.....	17.12	...do...	....	....	.....	....	....	....	....	....
1214	.....do.....	14.95	1½ optimums...	23.54	...do...	....	....	.....	....	....	....	....	....
1311	Orchard soil...	0	Air-dry.....	.28	...do...	....	....	.....	....	....	....	....	....
1312	.....do.....	4.47	½ optimum ...	8.46	...do...	....	....	.....	....	....	....	....	....
1313	.....do.....	8.93	1 optimum.....	15.39	...do...	....	....	.....	....	....	....	....	....
1314	.....do.....	13.41	1½ optimums...	21.37	...do...	....	....	.....	....	....	....	....	....
1411	Field soil.....	0	Air-dry.....	.14	...do...	....	....	.....	....	....	....	....	....
1412	.....do.....	3.48	½ optimum.....	6.63	...do...	....	....	.....	....	....	....	....	....
1413	.....do.....	6.95	1 optimum.....	12.32	...do...	....	....	.....	....	....	....	....	....
1414	.....do.....	10.41	1½ optimums...	17.34	...do...	....	....	[b] S.C.†.	....	....	....	....	....

[a] The writer did not think it advisable to include the remainder of Table III representing samples incubated at 5° to 7°, 22° to 24°, 32° to 33°, and at the outdoor temperature, as in no case were any living protozoa found during the period of eight days.

[b] S. C.†=few small ciliates.

Upon examining Table III it is seen that in but one sample of soil (the field soil which had an optimum and a half of moisture) were any active protozoa observed. It was noted that there was a little depression in the sample of soil and a little free available water was present, thus no doubt accounting for the presence of this organism on the third day of incubation, as on no other day and in no other soil were any motile protozoa seen.

In order to be certain that these soils contained cysts of protozoa and to collect some data as to the amount of moisture necessary for excystment and also to note the time of excystment of protozoa when conditions are favorable, to each series of the three different soil samples containing moisture to the amount of half optimum and optimum and a half sterile tap water was added to make the amount two optimums and two and one-half optimums, respectively. These samples were then incubated at the same temperatures as before, and daily examinations for a period of four days were made. (See Table IV.)

TABLE IV.—*Presence of active protozoa in different soils at different temperatures when the moisture conditions were favorable*

Laboratory No.	Kind of soil.	Moisture content.	Relative amount of moisture (in optimums).	Temperature of incubation.	Presence of active protozoa.[a]			
					8 to 12 hours after inoculation.	30 to 36 hours after inoculation.	Third day after inoculation.	Fourth day after inoculation.
		Per ct.		°C.				
1202	Greenhouse...	28.88	2	5 to 7.....	.....	.....	.....	F.†
1204	.....do.....	36.66	2½	5 to 7.....	.....	.....	.....	S.C.† F.†
1302	Orchard.....	26.52	2	5 to 7.....	.....	.....	.....	F.†
1304	.....do.....	29.05	2½	5 to 7.....	.....	.....	.....	F.†
1402	Field.....	21.83	2	5 to 7.....	S.C.†..	S.C.†.....	S.C.....	
1404	.....do.....	25.86	2½	5 to 7.....	F.†....	F.†.....	F.†.....	
1212	Greenhouse...	28.88	2	16 to 17.....	.....	.....	F.†.....	S.C.†
1214	.....do.....	33.66	2½	16 to 17.....	.....	.....	S.C.††† F.††.....	S.C.†
1312	Orchard.....	26.52	2	16 to 17.....	.....	S.C.†.....	S.C.† F.†.....	F.†
1314	.....do.....	29.05	2½	16 to 17.....	.....	F.†.....	S.C.†.....	S.C.† F.††
1412	Field.....	21.83	2	16 to 17.....	.....	S.C.†.....	S.C.† F.†.....	S.C.†† F.†
1414	.....do.....	25.86	2½	16 to 17.....	.....	F.†.....	F.††.....	F.†
1222	Greenhouse...	28.88	2	22 to 24.....	.....	.....	S.C.†† F.††.....	F.†
1224	.....do.....	33.66	2½	22 to 24.....	.....	S.C.† F.†..	S.C.†† F.††.....	S.C.†F.††
1322	Orchard.....	26.52	2	22 to 24.....	.....	.....	S.C.†.....	F.††
1324	.....do.....	29.05	2½	22 to 24.....	.....	S.C.†.....	S.C.† F.†.....	F.††
1422	Field.....	21.83	2	22 to 24.....	.....	S.C.†.....	S.C.†† F.††.....	S.C.†
1424	.....do.....	25.86	2½	22 to 24.....	.....	S.C.† F.†..	S.C.†.....	S.C.†
1232	Greenhouse...	28.88	2	32 to 33.....	.....	.....	F.†.....	S.C.†
1234	.....do.....	33.66	2½	32 to 33.....	.....	S.C.† F.†..	S.C.†.....	S.C.††
1332	Orchard.....	26.52	2	32 to 33.....	.....	S.C.†.....	S.C.†† L.C.† F.†	F.†
1334	.....do.....	29.05	2½	32 to 33.....	S.C.†..	F.†.....	S.C.†† F.†††.....	F.†
1432	Field.....	21.83	2	32 to 33.....	F.†....	F.†.....	S.C.††† L.C.† F.†	S.C.† F.†
1434	.....do.....	25.86	2½	32 to 33.....	.....	S.C.† F....	S.C.††.....	S.C.†††
1242	Greenhouse...	28.88	2	Outdoor temperature.	.....	.....	S.C.†.....	S.C.† L.C.†
1244	.....do.....	33.66	2½	.....do.....	.....	.....	S.C.†.....	F.†
1342	Orchard.....	26.52	2	.....do.....	.....	S.C.† F.†..	S.C.†† F.†.....	F.†
1344	.....do.....	29.05	2½	.....do.....	.....	S.C.†.....	S.C.† F.††.....	S.C.† F.††
1442	Field.....	21.83	2	.....do.....	.....	S.C.† F.†..	S.C.† F.†.....	L.C.† F.†
1444	.....do.....	25.86	2½	.....do.....	.....	F.†.....	S.C.† F.†.....	S.C.† F.††

[a] S.C.=small ciliates, L.C.=large ciliates, F.=flagellates, A.=amebæ, †=few, ††=several, †††=many.

The data presented in Tables III and IV again point to the fact that the supply of sufficient moisture is the limiting factor which influences the presence of protozoa in the active state in the soil, while the temperature, the presence of organic matter, and the soil structure seem to be only secondary factors.

On examining Table IV it becomes apparent that the temperature influences the period of excystment, in that a higher temperature may encourage a more rapid excystment of a greater number of protozoa and that the physical character of the soil may be more or less influential in the movement of the organisms in the soil; yet if the moisture content is not high enough, the protozoa will not be present in the active state.

To find out whether protozoa were always present in the active state in water-logged soils, samples of six soils, three greenhouse and three field soils, which were kept in the laboratory for some time, were put into small bottles, water-logged, and the bottles plugged with rubber stoppers to prevent evaporation, and then allowed to stand in the labo-

ratory. Examinations were made from time to time for a period, and then the samples were placed outside in the open air where the temperature variation was great and examinations were again made. (See Table V.)

TABLE V.—*Presence of active protozoa in water-logged soils, under constant and variable temperatures*

Laboratory No.	Kind of soil.	Presence of protozoa when incubated at room temperature on—[a]		
		May 25.	June 4.	June 7.
1501	Greenhouse	S.C.† L.C.†	S.C.††† L.C.† F.†††	S.C.† L.C.†
1502	do	S.C.††† L.C.†† F.††† A.†	S.C.††† F.††	S.C.††† F.††
1503	do	S.C ††† F.††	S.C.†† F.††	S.C.††† F.†
1504	Field	S.C.††† L.C.† F.††	S.C.†† F.††	S.C.†††. F.†††
1505	do	S.C.††† F.†	S.C.†††	S.C.†††
1506	do	S.C.††† A.†	S.C.††† L.C.† F.†	S.C.†††

Laboratory No.	Kind of soil.	Presence of protozoa when incubated at outdoor temperature on—[a]		
		June 8.	June 16.	June 23.
1501	Greenhouse	S.C.† L. C.†	S.C.† L.C.† F.†	S.C.† F.†
1502	do	S.C.††† F.†	S.C.††† L.C.††	S.C.† F.†
1503	do	S.C.†††	S.C.†††	S.C.†
1504	Field	S.C.††† L.C.† F.†	S.C.††† L.C.††	S.C.† F.†
1505	do	S.C.†††	S.C.†††	S.C.†† F.†
1506	do	S.C.†† L.C.†	S.C.††† F.††	S.C.†† F.†

[a] S. C.=small ciliates; L. C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

The data given in Table V indicate that living protozoa were always present in all of the water-logged soils during incubation at outside temperature as well as at room temperature. It was noted that the sudden change from the room temperature to the outside temperature did not have any marked effect upon the existence of the organisms in the active condition.

PERIOD OF EXCYSTMENT OF SOIL PROTOZOA

Since active protozoa were not found in normal field soils, the question at once presented itself, How long a period of time was required for soil protozoa to become active in the presence of sufficient moisture, as, for instance, during a heavy fall of rain, and How long will they remain in the active state? In his work with *Colpoda cucullus* Goodey (3) in 1913 found that at 30° C. many were active after an hour. It was suggested by Martin and Lewin (8), as previously noted, that they may become active in a few minutes. To prevent misunderstanding as to the presence of motile protozoa in the soil, the writer in his method of examination proposed a 2-minute examination of each sample—i. e., the soil was in contact with free water no longer than two minutes at each examination. In no case during the entire course of the many examinations of field soils were any protozoa noted to have excysted

during the 2-minute examination, for in no case were any living protozoa found. It was later found with a limited number of soils examined that no protozoa were observed to excyst in a 5- or even 7-minute period. More evidence on this point is being collected.

Some evidence as to the length of time required for the excystment of soil protozoa when sufficient moisture is available is presented in Tables II and IV. As shown in Table IV, at the incubation temperatures of 5° to 7° and 32° to 33° a few small ciliates and flagellates were observed 8 hours after the increased additions of water were made to the soils. It is also seen that in nearly all samples incubated at 15° to 17°, 22° to 24°, 32° to 33°, and at outdoor temperatures some motile protozoa were present after 30 hours. The higher temperatures seemed to be more favorable for the more rapid excystment. This was also found to be true (1) when protozoa were developed in artificial-culture solutions. Small ciliates excysted in as short a period as did the flagellates. In Table II it is shown that after the soils had been in contact with water for 40 hours all of them showed the presence of small ciliates and flagellates. In several samples active large ciliates were also observed.

In order to accumulate more data as to the period of excystment of protozoa a small sample of each of the three soils (samples air-dried and samples containing an optimum amount of moisture and incubated at 22° to 24°, as given in Table III and in the text just following Table III) were added to a few drops of sterile tap water on a glass slide with a large depression in the center. The soil was stirred with a stirring rod and the film spread over the surface of the slide. A careful examination of each sample was made for a period of five minutes, and the slides containing the samples were then placed in the incubator. They were again examined for 5-minute periods at intervals of 15 minutes and 1, 2, 3, 5, 6, and 8 hours. (See Table VI.)

TABLE VI.—*Time required for the excystment of soil protozoa at 22° to 24° C.*

Laboratory No.	First examination.	After 15 minutes.	After 1 hour.	After 2 hours.	After 3 hours.	After 5 hours.	After 6 hours.	After 8 hours.
1221	.....	.....	.....	.....	.....	.....	.....	F.††
1223	.....	.....	.....	.....	.....	.....	F.†	.....
1321	.....	.....	.....	.....	.....	.....	.....	.....
1323	.....	.....	S.C.†	S.C.†	.....	.....	S.C.†	.....
1421	.....	.....	.....	.....	.....	.....	.....	.....
1423	.....	.....	.....	.....	.....	.....	.....	.....

[a] S. C.†= few small ciliates; F.†=few flagellates; F.††=several flagellates.

From the data recorded in Table VI it will be noted that at 22° to 24° protozoa (small ciliates) may excyst within two hours after the protective cysts come in contact with available moisture. Flagellates and other

small ciliates are seen to excyst in from six to eight hours after the immersion of the cysts in water. From the limited amount of study given to this point no conclusive statement as to the relative length of time required for the excystment of soil protozoa can be made. Nevertheless, the writer is of the opinion that under normal conditions protozoa excyst seldom, if at all, in as little as two minutes. There may be cases, however, as where the protective cyst is partially ruptured either by mechanical means or otherwise or where the moisture conditions are almost favorable enough for excystment, in which the organisms will become active in less than two minutes; but under ordinary normal conditions it seems doubtful from the examinations already made whether they can become active in this period of time at 22° to 24°. The indications (Table IV) are that excystment goes on more rapidly at higher temperatures. In all probability the original moisture content of the soil plays a part in determining the length of time which must elapse before the organisms become active. Likewise, different types of protozoa will prefer different conditions (1) and may excyst sooner at one temperature than at another. Further study on this point will be made.

SUMMARY

Under the conditions recorded in this paper the following observations as to the activity of soil protozoa seem to be justified:

(1) Under ordinary greenhouse conditions small ciliates, flagellates, and amebæ are active in some soils, but their presence is very limited.

(2) Active protozoa (small ciliates, large ciliates, flagellates, and amebæ) do not seem to be present in field soils with a normal moisture content and even when the moisture content is slightly supernormal, and, hence, they would not be a limiting factor in the soil.

(3) All field soils contain cysts of protozoa the organisms of which become active when conditions become favorable.

(4) The moisture content of the soil is the primary influencing factor which determines the presence or absence of the active protozoa in the soil, while the temperature, the presence of organic matter, and the physical properties of the soil are secondary factors.

(5) Soon after standing water is accumulated, as after a heavy rain, some protozoa will excyst and be active as long as the moisture content is favorable. Active protozoa seem to be always present in free standing soil water.

(6) Active protozoa are present in water-logged soils at constant and variable temperatures.

(7) Under normal conditions it would seem that protozoa can not excyst in 2 minutes. Small ciliates can excyst in 1 to 2 hours at 22° to 24° C.; at the same temperature flagellates can excyst in 6 to 8 hours and large ciliates can excyst in 40 hours.

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BERIBERI AND COTTONSEED POISONING IN PIGS[1]

[PRELIMINARY NOTE]

By GEORGE M. ROMMEL, *Chief, Animal Husbandry Division, Bureau of Animal Industry*, and EDWARD B. VEDDER, *Captain, Medical Corps, United States Army*

SO-CALLED COTTONSEED POISONING OF ANIMALS

Cottonseed meal is one of the most valuable feedstuffs at the command of the American stockman. After the animal has digested it, the value of the residue as fertilizer is about three-fourths the original value of the meal. The United States uses only part of the cottonseed meal which it produces, and one of the reasons which prevent a larger domestic consumption of this by-product of the cotton industry is the danger that sickness and death may follow its use.

Cattle fed for more than 90 to 120 days on a heavy cottonseed-meal ration (6 pounds or more per head daily) become lame, and their eyes discharge freely, blindness often resulting. Deaths may occur, especially in young animals. Pigs are peculiarly susceptible to the effects of cottonseed meal, possibly because they are usually fed a larger quantity of the meal in proportion to their body weight. In feeding pigs, symptoms of sickness may appear at any time after three weeks of feeding, and deaths frequently occur with little warning.

Various systems of feeding cottonseed meal to pigs have been devised. Some of them appear to minimize its danger somewhat, but none of them prevent it entirely. This product, therefore, can not be regarded as a safe feed for pigs in the combinations in which it has heretofore usually been fed.

Among the more pronounced symptoms observed in pigs suffering from the effects of cottonseed-meal feeding are diarrhea; a harsh, rough, curly coat; paralysis; and shortness of breath. Emaciation and dropsical conditions are frequently observed. The disease manifests two forms—acute or chronic.

The acute form is much more serious to the farmer, because pigs are attacked by it with little warning and may be dead before any indications of disease are noticed. The largest and best nourished pigs are often the ones attacked. The attack is sudden and sharp. The pig experiences extreme shortness of breath and suffers the most intense pain. If he recovers, recurrences of the attack are likely, especially if the pig is a heavy feeder. Subsequent attacks may end fatally, or the disease may assume the chronic form.

[1] This opportunity is taken to express appreciation of the cooperation of Dr. Adolph Eichhorn, Chief of the Pathological Division of the Bureau of Animal Industry, in having made the necessary post-mortem examinations of pigs used in these experiments.

Journal of Agricultural Research,
Dept of Agriculture, Washington, D. C.
bq

Vol. V, No. 11
Dec. 13, 1915
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In the chronic form fatal results may not occur for a considerable time. The symptoms persist if the feed is not changed, and the pig appears to develop a certain degree of immunity to the effects of the disease. His condition, however, is continually, although slowly, declining. Pigs suffering from this form of the disease may live for a year or more on a cottonseed-meal ration.

On post-mortem examination, pigs which have died from the effects of cottonseed-meal feeding show large quantities of fluid in the abdominal and thoracic cavities and in the pericardial sac. The kidneys, liver, spleen, and small intestines are usually congested. In some cases the membrane lining the stomach is eroded. The lungs are very edematous, especially in pigs which have died from sudden acute attacks. The heart is enlarged.

SIMILARITY OF SYMPTOMS OF COTTONSEED POISONING AND OF BERIBERI

These conditions bear a striking resemblance to those seen in the disease known as beriberi in man, which, according to Vedder,[1] results "from faulty metabolism * * * and is directly caused by the deficiency of certain vitamines in the food."

Beriberi in human beings is usually caused by a diet of highly milled rice and is never known to result from a diet of rice from which the pericarp and aleurone layer of the grain have not been removed. However, the disease may be caused by diets of which rice forms no part whatever. For example, a diet of bread or macaroni alone made from highly milled wheat flour will produce beriberi. Birds (chickens and pigeons) are generally used in the laboratory study of beriberi because they readily develop the chronic or "dry" form when fed on a diet of highly milled rice for a sufficient time, but they will also develop the disease if fed on an exclusive diet of white wheat bread.

Beriberi in pigs is not frequently reported in the literature on the subject. Braddon[2] reports, without details, the case of a pig fed on polished rice. The pig developed paralysis in about a month and died suddenly. It is believed that until this year this was the only case of the kind recorded.

EXPERIMENTS TO COMPARE EFFECTS OF FEEDING POLISHED RICE AND COTTONSEED MEAL

On August 31, 1915, the writers began a series of experiments to determine (*a*) whether the "wet" or acute form of beriberi could be produced in pigs on a diet of polished rice, and (*b*) whether the disease heretofore called "cottonseed poisoning" in pigs is not really beriberi.[3] Four pigs

[1] Vedder, E. B. Beriberi. p. viii. New York, 1913.

[2] Braddon, W. L. The Cause and Prevention of Beri-Beri. p. 355. London, New York, 1907.

[3] It should be noted that Withers and Carruth made no extensive use of pigs in their investigations on gossypol. (Withers, W. A., and Carruth, F. E. Gossypol, the toxic substance in cottonseed meal. In Jour. Agr. Research, v. 5, no. 7, p. 261–288, pl. 25–26. 1915.)

were fed a ration of 9 parts (by weight) of steamed polished rice and 1 part of tankage, and four a ration of 2 parts of corn meal and 1 part of cottonseed meal. On October 24 the ration of the latter pigs was changed to equal parts by weight of corn meal and cottonseed meal. None of these pigs had received rice or cottonseed meal before they entered the experiment.

On September 8 one of the pigs on rice began to breathe with difficulty. On the 10th this condition was pronounced, and he refused to eat. On September 14 these symptoms rapidly became more severe, paralysis developed, and the pig died shortly before noon. The ante-mortem symptoms were what one would expect to see in an acute case of so-called cottonseed poisoning. They were, in fact, the symptoms of wet beriberi. The post-mortem examination showed serous fluid in the pericardial sac and in the thoracic and abdominal cavities. The heart was enlarged and the cardiac muscle congested. The lungs were decidedly edematous and mottled with a fair number of small subpleural hemorrhages. The liver was intensely congested and enlarged. The spleen was apparently unaltered, but was dark in color. The stomach showed several erosions in the mucosa, and the walls were thickened. The small intestines were slightly congested. Many of the mesenteric glands were enlarged and congested. Both kidneys were congested, especially at the apices, which were deep cherry-red in color. The bladder was distended with urine, which contained a large amount of albumin. Except for the large quantity of albumin, this is exactly what one would expect to find in a beriberi necropsy. It is also what is found in an acute cottonseed-meal necropsy.

On September 21 four additional pigs were placed on the same steamed rice and tankage ration (9:1). On September 29 one of these pigs became sick and on September 30 it refused to eat. He recovered and regained his normal appetite, but died on October 29, after having been on the rice diet for 38 days. The ante-mortem symptoms corresponded closely to those of the first pig to die, but the post-mortem examination did not give such clear-cut results. The sciatic nerves of this pig were dissected out immediately after the post-mortem examination and, after being treated by the Marchi method, showed considerable degeneration of the nerve fibers.

The writers believe that pigs fed a ration in which rice is the chief component will develop beriberi as do human beings, but much more quickly. Weight is given to this belief by the experience of Moore,[1] who lost pigs fed on "rice meal" [2] from a disease which Hadwen [3] suspects to be beriberi.

[1] Moore, P. H. Hog-feeding experiments. In Canada Exp. Farms Rpts. [1912]/13, p. 611–613. 1914.
——— Preliminary note on the effects of feeding rice meal to pigs. In Canada Dept. Agr. Rpt. Vet. Dir. Gen. [1913]/14, p. 137–141. 1915.

[2] Apparently not the rice meal of our Southern States.

[3] Hadwen, S. Notes on the pathology and symptoms of rice-meal fed pigs. In Canada Dept. Agr. Rpt. Vet. Dir. Gen. [1913]/14, p. 140. 1915.

The remaining 10 pigs are being continued on the rice and cottonseed-meal rations. At the time this article is written they have been almost 90 days on these feeds. All the pigs are sick, and the same symptoms have appeared in each lot. In fact, it may be said that the most typical and acute cottonseed-meal symptoms are seen among the pigs receiving rice.

A mature brood sow, weighing 400 pounds, due to farrow on November 14, 1915, was placed on a cottonseed-meal ration on September 2. She was started on a ration of 4 parts of corn meal and 1 part of cottonseed meal, the quantity of corn meal being gradually decreased until, on October 1, she was receiving equal parts of corn meal and cottonseed meal. Up to November 14 she had eaten 134.65 pounds of cottonseed meal. She showed no serious sign of sickness, except nausea on November 4, when she vomited. At 8 p. m. on November 13 she began to farrow and delivered 9 pigs, the last one being born at 4 o'clock the following morning. Four of these pigs were born dead, and of those born alive all but one died in a few minutes. The last pig born lived less than eight hours.

Post-mortem examinations were made of seven of these pigs, four of which had been born alive. All of them showed enlarged hearts, and serum was found in the pericardial sac, the thoracic cavity, and the abdominal cavity. The quantity of serum was a little greater in the pigs born alive than in those born dead. In the pigs born alive there was some injection in the lungs, liver, and small intestines, but none in those born dead. There were no alterations in the kidneys of any of the pigs born alive or dead.

These pigs were very well developed, plump, and apparently had been well nourished. They averaged slightly over 2 pounds 6 ounces in weight. The analogy with infantile beriberi is apparent. Yet the dam had never eaten rice, and the only assignable cause for the death of her litter was the cottonseed meal in her ration. Her breeding record for previous farrowings is as follows:

Item.	1914	1915
Date of farrowing	Apr. 7	Apr. 2
Number of pigs	5	12
Number born alive	5	9
Number raised	4	5

The sow was a good breeder, and difficult labor can not be given as the cause of the death of the litter.

CONCLUSIONS

The studies of the writers seem to lead to three general conclusions:

(1) Pigs are susceptible to beriberi when fed on vitamine-deficient rations, such as rice. The disease develops much more rapidly in pigs

than in man. In man symptoms rarely, if ever, appear before 90 days. In pigs the writers have found symptoms of a pronounced character in from 8 to 10 days.

(2) It is believed that the so-called cottonseed poisoning of pigs is a deficiency disease, analogous to the disease known as beriberi in man, if not indeed identical with it. Acute cottonseed poisoning corresponds to wet beriberi, and the chronic form to dry beriberi.

(3) The cause of the so-called cottonseed poisoning is probably a deficiency in the ration, causing, among other manifestations, profound changes in the nervous system.

At first thought this theory is not justified. Beriberi results from a ration of highly milled rice, because substances vitally necessary to the animal organism have been removed from the rice grain in the process of milling. When pigs suffer from so-called cottonseed poisoning, it is only when cottonseed meal has been added to the ration. Pigs are seldom, if ever, fed on cottonseed meal alone.

The following explanation of this condition is offered: The grain with which the cottonseed meal is most frequently combined is corn. Corn is notoriously deficient as a single feed for animals, and it must be properly balanced to be fed satisfactorily. The excellent results in feeding pigs which can be obtained from rations of corn meal and skim milk or other animal products, such as tankage, blood meal, fish meal, etc., are out of all proportion to the facts indicated by the conventional chemical analyses of protein, carbohydrates, and fat. When corn meal is fed with cottonseed meal, a combination is made of two feeds both of which are deficient.

The writers are engaged in further studies of this subject to determine more exactly the effects of cottonseed meal when fed in the ration of the pig, and to determine whether methods similar to those used to prevent beriberi in man can be practically applied to prevent the so-called cottonseed poisoning of pigs.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., DECEMBER 20, 1915 NO. 12

BIOLOGY OF APANTELES MILITARIS

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INTRODUCTION

The results herewith presented deal with *Apanteles militaris* Walsh, a braconid endoparasite of the army worm (*Heliophila unipuncta* Haw.). The series of experiments on which the main part of this paper is based was begun on September 29, 1914, at La Fayette, Ind. They were carried on in the laboratory, the parasitized caterpillars being kept in glass vials plugged with cotton and fed fresh corn leaves as required. The laboratory windows were left open, so as to make conditions as nearly like those outside as possible. During the few cold days which were experienced the laboratory was heated to the normal room temperature. During the first two weeks in August additional records were kept of the time spent in the cocoon by the parasites, and in these experiments cocoons were kept in tin salve boxes in an outdoor insectary. On November 16 a series of experiments was started indoors to determine whether or not this species is parthenogenetic, and conclusive results were obtained. The caterpillars used in the experiments were raised from eggs unless otherwise stated.

DESCRIPTION OF LIFE STAGES

THE EGG

The egg measures 0.09 to 0.10 mm. in length and 0.025 to 0.028 mm. in width. It is rounded at one end, more or less pointed at the other, and slightly curved, the rounded end bearing a distinct micropyle. Subsequent swelling of the egg during the growth of the embryo causes

[1] The writer wishes to acknowledge his indebtedness to Messrs. J. J. Davis and A. F. Satterthwait, of the Cereal and Forage Insect Field Station of the Bureau of Entomology at La Fayette, Ind., for many helpful suggestions and material, and to Messrs. J. A. Hyslop and G. G. Ainslie, of the Bureau of Entomology, for their interest and kindness in collecting material and data for him at their respective stations. He is also indebted to Messrs. A. B. Gahan and W. R. Walton, of the Bureau of Entomology, for the determination of specimens and for the drawings of the three larval stages, respectively.

the point of the smaller end to assume the appearance of a nipple-like prominence.

The number of eggs laid by a single individual was not obtained, nor were the eggs in the abdomen counted, hundreds having been present.

EMBRYONIC DEVELOPMENT

The average length of the egg stage is 5½ days. Individual records show that in some cases this may be shortened to 4¾ days or prolonged to more than 6⅛ days. From the hundreds of developing eggs examined it was determined that only one larva hatches from each egg.

Development progresses rapidly within the egg. At first little can be distinguished, except that the egg becomes strongly curved, increases in size, and becomes more opaque, owing to the formation of the germ band. When the egg is ready to hatch it has increased in size from 0.09 or 0.10 mm. in length to 0.66 or 0.70 mm., and proportionally in width. This great increase in size can possibly be explained by the fact that the egg is probably deficient in nutritive matter when laid and that this is absorbed from the blood of its host by the developing embryo.

When embryonic development has progressed sufficiently to show the form of the embryo, this is seen to be surrounded by a single embryonic envelope one cell layer deep which, according to Korschelt and Heider (3, p. 287),[1] is the serosa (Pl. L, fig. 1). Whether the amniotic and serosal envelopes are at first separate has not been determined. According to Graber's observations on Hymenoptera, as reviewed by Korschelt and Heider, it would seem that the two envelopes are separate at first but later become indistinguishably united. At the time of hatching, a portion of the cells of this so-called serosal envelope are cast out at the poles of the egg (Pl. L, fig. 2) and become a body of loose cells lying between the chorion and the embryo (Pl. L, fig. 3), which is now tightly inclosed by a layer of broad, flattened cells made up of the remaining cells of the envelope (Pl. L, fig. 3). This rapid division apparently indicates that this envelope was the product of the fused amnion and serosa, which now separate at hatching time, the loose mass of cells being of serosal origin and the remaining thin envelope the amnion surrounding the embryo. Henneguy (2, p. 336–337), however, discusses insects that have only one embryonic envelope and lists among these parasitic forms, vegetable or animal, of the Cynipidae, Pteromalidae, and probably Ichneumonidae. It will be interesting to note whether other investigators observe this splitting of the single embryonic envelope at hatching time.

The mandibles can be seen forming at an early stage, and their chitinization can be seen to progress until maturity is reached at hatching time.

[1] Reference is made by number to "Literature cited," p. 506–507.

The mouth opens into an enlarged cavity, the pharynx, this in turn opening posteriorly into a very narrow esophagus, and this into the stomach, which is a very long, narrow, tapering tube closed posteriorly. There are two Malpighian vessels, which lie parallel to the stomach, extending anteriorly about one-half the length of the larva.

The tracheal system has not been observed in the embryo. According to the observations of Weismann and Grasse, as reviewed by Korschelt and Heider (3, p. 334–335), the tracheal system forms early in the embryonic development of the Hymenoptera as compared with the lower forms of insects and usually contains air previous to hatching, this being obtained apparently from its tissues and body fluid. Seurat (7) states, however, from his study of *A. glomeratus*, that the tracheal system of this parasite, whose development is similar to that of *A. militaris*, is present, although he had not seen it, no doubt basing his statement on the fact that these organs, being ectodermal invaginations, are normally formed in the embryo.

The head of the mature embryo is of one segment and is readily distinguished by its large size, the presence of mandibles, two small tubercle-like antennæ, and the prominent brain lobes. A nervous system of 11 ganglia, not including the subesophageal ganglion, is visible. The segments of the body appear to be 10 in number, but subsequent development and growth in the first stage reveal 11 distinct segments.

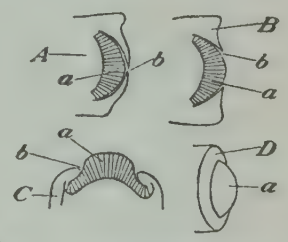

FIG. 1.—*Apanteles militaris*: *A*, *B*, *C*, Diagrammatic sectional views of the posterior end of the embryo, showing how the hypertrophied cells of the hind gut, which ultimately form the caudal vesicle, grow out through the anus. *D* shows an external view of this process. *a*, Mass of cells; *b*, anus. (Original.)

The caudal vesicle, which in the larval forms is a large sac at the end of the body, is seen forming as a solid mass of long, narrow cells in the posterior region of the abdomen (fig. 1, *a*). When first seen it lies inside the abdomen, but can be seen gradually to grow out through the anal opening (fig. 1, *b*), which becomes greatly distended. The stomach becomes lengthened and extends outside the body into the vesicle, its blind end being fastened to the inside wall of the vesicle posteriorly and ventrally. The Malpighian tubes also extend into the vesicle and open through its ventral surface near the end of the stomach.

HATCHING

The embryo at the time of hatching, as previously stated, lies tightly inclosed in the amniotic envelope surrounded by the loose mass of serosal cells, the whole being surrounded by the chorion. The embryo, which up to this time has been curled in the egg, now straightens itself out and by its struggles to escape, aided by the rapid swelling of the serosal cells, ruptures the chorion, which has become extremely thin, owing to the increase in the size of the egg, and escapes into the body of its host, still

tightly inclosed in the layer of thin, flat cells. The serosal cells are scattered through the body of the host. The chorion shrinks and probably finally dissolves. The young larvæ are now 0.7 mm. in length and start feeding, after cutting through the amnion in the mouth region. At this time the mass of cells which forms the caudal vesicle has grown out through the anal opening.

THE LARVA

FIRST INSTAR (Pl. L, fig. 5).—The first larval instar averages 3¼ days, the first molt taking place, on an average, 8¾ days after oviposition.

The larva grows rapidly, increasing in length approximately from 0.7 mm. at hatching to 3.5 mm. at the first molt.

The head is made up of 1 segment and the body appears to have 10, but in subsequent growth the tenth segment divides into 2, making 11 in all. There are no spines or hairs on the segments, except a few in the oral region. Owing to the rapid growth of the larva, the embryonic envelope in which it is inclosed becomes ruptured and gradually falls off, although portions of it may remain until the first molt takes place. The mandibles are constantly in motion, attacking the fat body of the host. This, together with the blood, is the food of the parasites during this stage and is drawn in by means of a sucking pharynx. The alimentary tract does not change, except to increase in size, it being still further lengthened as the caudal vesicle expands.

Immediately following hatching, the slender cells of the mass which protrudes from the distended anal opening are compressed lengthwise, so that they become broad, flat cells, thus immensely increasing their exterior and interior surfaces, and there is formed at the end of the larva a large sac, the caudal vesicle, the walls of which are made up of a layer of broad, thin cells (Pl. L, fig. 5). The two Malpighian vessels are drawn out into the caudal vesicle, their relative positions being the same as in the embryo.

The origin of this caudal vesicle and its functions in the two endoparasitic stages will be considered later.

The nervous system appears as in the embryo, its growth keeping pace with the growth of the larva.

No tracheal system is visible during this instar.

The heart can be seen forming in the early part of this instar. It lies dorsally and has nine pairs of valves, its lateral controlling muscles being readily seen. Anteriorly it narrows to an aorta which opens into the posterior region of the head. Instead of ending normally in the posterior end of the body, a rudimentary tube lying dorsally in the caudal vesicle connects with the heart (Pl. L, fig. 4). This tube extends posteriorly, opening in the dorsal posterior region of the caudal vesicle, and forms a channel through which the blood is sucked into the heart. When the heart commences to function, which it does during this stage, the blood,

having been drawn through the rudimentary tube into the heart, is there passed along by a series of wavelike motions into the head, the valves preventing the return of the blood. From here it circulates through the body in returning to the caudal vesicle, the walls of which it bathes before starting on a new cycle. A careful examination of the heart does not show that ostia are present; hence, the blood necessarily follows the course described above.

The silk glands can be distinguished early in this stage and lie on either side of the stomach as two straight tubes which meet anteriorly in the head and extend to the spinneret. As the end of this stage approaches, these glands begin to coil, taking on a wavy appearance.

SECOND INSTAR (Pl. L, fig. 6).—The second instar averages $5\frac{1}{12}$ days, terminating when the larva emerges from its host, for it molts at this time. During this stage the average increase in length is from 3.5 to 6 mm., although when a great many larvæ are present in a host their size may be reduced nearly one-half. The caudal vesicle normally during this stage reaches the length of 1 mm. (Pl. L, fig. 6).

The head of the larva is made up of 2 segments. The anterior one bears a few spines about the oral region and is much smaller than the posterior and almost wholly retractile in it. There are no notable characters or ornamentations on the segments of this larva. The body has 11 segments and is at first slightly darker than the first instar, but rapidly becomes more so as the fat body accumulates. The mouth parts are not developed, nor are those of the third instar ready for use, until the larva is ready to emerge from its host; hence, it is seen that only the blood and the solid matter contained in it are used for food during this stage. In older forms there are 7 hyaline areas protruding on each side of the body lying between the segments.

The silk glands grow rapidly, becoming more and more coiled and twisted, and are readily seen lying on either side of the alimentary tract, nearly filling the body cavity.

The heart and the circulation of the blood are the same as in the first instar.

The nervous system consists of the supraesophageal and the subesophageal brains and 11 ganglia with their branches, as in the first instar. In the early life of this stage the imaginal discs of the compound eyes are noticeable and appear to be in the first thoracic segment. The exhaustive studies of Seurat (7) show clearly that although other authors have thought that a portion of the prothorax entered into the composition of the head of the pupa, it is formed only from the head of the larva and that in the larval forms a portion of the head has simply been thrust back into the prothorax. Ventrally in the thoracic segments the three pairs of imaginal discs of the legs are present, and laterally in the mesothorax and metathorax those of the wings can be seen.

The mouth, pharynx, esophagus, and stomach have approximately the same form and relative positions as in the first instar. Owing to the fact that the blood of the host is green, the stomach content of the parasite at first takes on a greenish brown color which finally becomes a deep green, similar to the blood of the host, and later, at the end of the stage, this again becomes greenish brown.

During the last two days of this stage the anal opening, the diameter of which nearly equals that of the body, slowly contracts, and violent contractions of the longitudinal muscles of the stomach, which cause it to shorten, slowly draw the caudal vesicle in through the contracting anal opening. The Malpighian vessels are also drawn in by the contraction of the stomach and are now two-thirds as long as the larva. After the caudal vesicle has been drawn completely within the body, the anal opening contracts still further, and the anus is formed.

The tenidia of the tracheal system can be seen forming soon after the first molt. Those of the two main longitudinals and their anterior connecting branches are first visible, and there are 11 branching centers on each longitudinal from which arise branches sending tracheæ to all parts of the body, some even extending posteriorly into the caudal vesicle along the lateral walls and the stomach. Nine pairs of short, stublike branches are noticeable in the older larvæ, arising near the bases of the anterior nine pairs of dorsal branches of the main longitudinals. In the still older larvæ, those nearly ready to emerge, eight pairs of spiracles can be seen forming at the surface of the body, and these are connected with the first, and the third to ninth, inclusive, pairs of stublike branches previously mentioned, by tracheæ destitute of air. These become filled with air when the larva molts at emergence and the spiracles are uncovered and function. The spiracles that connect with the second pair of stublike branches do not form during this stage.

After the caudal vesicle has been drawn in, the larva is ready to emerge from its host. The mandibles of the third instar, which are now developed and protrude, slowly cut and tear through the muscles and skin of the host as the larva presses its head against the body walls of the caterpillar and moves them backward and forward. When a slit has been made of sufficient size the larva squeezes through the opening, molting the previously loosened skin as it emerges. During this process the caterpillar lies quietly as though paralyzed. About the time the parasites have nearly finished their cocoons, it usually revives enough to crawl away.

THIRD INSTAR (Pl. L, fig. 7).—The third instar lasts from the emergence from the host until pupation, the time being approximately 2½ days.

The newly emerged larva is light green in color. It is covered with minute spines, with a number of short black spines somewhat irregularly

placed on the segments; also about the mouth there are a few hyaline spines. The brown-colored compound eyes are very noticeable and appear to be, as in the second stage, in the prothorax. The segmentation of the body is apparent and the eight pairs of spiracles are plainly visible (Pl. L, fig. 7). The mouth parts are at the extremity of the head and are composed of labrum, mandibles, maxillæ (bearing the rudimentary maxillary palpi), and the labium (bearing the rudimentary labial palpi). Laterally the seven hyaline protruding areas form an irregular, conspicuous, longitudinal ridge on either side of the body.

When the larva has emerged for about two-thirds of its length, it stops and commences to spin its cocoon. The silk comes from the two orifices of the spinneret situated at the base of the labium. The cocoon is spun in two parts, the outer part loosely and the inner compactly. The first few threads spun are fastened to the ventral side of the body, after which a series of large loops are made, the silken thread being drawn out and fastened to the top of the loop below. These extend up the ventral side laterally and over the head of the larva as far back as it can bend. The larva now draws its anal end out of the host, reverses its position in the partly spun outer cocoon, and spins the remaining side and end. The inner or thin, dense cocoon is now spun by a series of long, narrow, longitudinal and diagonal loops. The tough silken cocoon is encircled near one end, or sometimes at both, by a thinner, narrow area, through which the adult parasite easily cuts, removing a caplike portion, the end of the cocoon, as it emerges.

At the end of the first day or the beginning of the second the connection between the stomach and proctodeum is opened and the accumulated waste is voided, being deposited at the anal end of the cocoon. When pupation takes place, the last larval skin is molted and pushed to the anal end of the cocoon and lies over the waste. Previous to pupation, the constriction between the thorax and abdomen, which results in the cephalization of the first abdominal segment, is distinctly seen.

PUPA AND ADULT

The pupal stage averages from 8½ to 9½ days.

The pupa is light cream yellow and lends the same color to the cocoon. The eyes and ocelli appear as brown spots. Later, the chitin in the head and thoracic region commences to darken, closely followed by that of the abdomen. When the adult becomes active in the cocoon, the pupal skin is kicked off, and the area of thin silk is cut through by the mandibles, the end, or cap, of the cocoon being pushed off by the emerging adults. As soon as the adult is out of the cocoon, it passes a quantity of waste, cleans itself, and straightens and dries its wings.

LENGTH OF LIFE CYCLE

The total length of the life cycle, as obtained in the series of experiments carried from the last of September to the last of October, averaged 25 days. A series of experiments conducted during the first two weeks of August to determine the time spent by the third instar and pupa in the cocoon varied from 5 to 7 days, as compared with 11 to 12 days during September and October. This great reduction in the time spent in these periods of development raises the question whether or not the time spent in the host would not be shortened under summer conditions. Unfortunately, this point could not be determined; but considering that the duration of the larval life of the army worm varies from 20 to 30 days, according to Slingerland (8), it seems not unlikely that the length of the egg and internal larval stages would vary correspondingly with the life of the host.

COPULATION

The following observations were made on these insects confined in test tubes and lantern-globe cages. The male pursued the female, caressing her with his antennæ, often mounting her posteriorly and, thrusting his abdomen forward, bringing the ventral surface in contact with that of the female. Once union had taken place the male folded his wings and drew his legs close to his body, holding on to the female solely by his genitalia. It was noticed that in the case of a number of males and females confined in test tubes for several days, copulation continued to take place day after day with unabated vigor.

OVIPOSITION

The parasite apparently recognizes the host on touching it with its antennæ, and following such recognition the ovipositor is bent beneath the thorax, sometimes slowly but usually quickly, and is then rapidly thrust into the caterpillar. This being done, the parasite folds its wings and draws its legs up close to its body, holding on to the caterpillar solely by its ovipositor, this no doubt being done to protect itself from the attacks of its host. During the process of oviposition the caterpillar may throw itself about violently, but rarely dislodges the parasite.

Of the number of apparent ovipositions in larvæ of the third, fourth, and fifth stages, one-sixth of those which took place in the third, one-fifth of those in the fourth, and one-half of those in the fifth stage were unsuccessful. Usually the parasite larvæ emerge after the caterpillar is full grown, as observed in the case of larvæ collected in the field and those parasitized in the laboratory under artificial conditions, but in one instance where the parasite oviposited in a caterpillar of the third stage the parasite larvæ issued during the fifth stage.

Parasites readily attempted to oviposit in caterpillars of the fifth and sixth stages, but were apparently unsuccessful, on account of the tough-

ness of the skin, except in newly-molted fifth-stage larvæ. In such cases they would run along the back of the host, jabbing with the ovipositor but never succeeding in puncturing the skin.

The eggs, when dissected from the body of a caterpillar immediately following oviposition, are found to be separate.

Oviposition in the field under natural conditions resulted in the following numbers of cocoons collected from single hosts: 56, 90, 71, 79, 90, 7, 113, and 66. In the laboratory from 8 to 72 eggs were deposited in one oviposition of less than one second, and in one case of four ovipositions 210 eggs were deposited in the same host. The extreme rapidity of oviposition is apparently due to the activity of the caterpillar, which usually immediately recognizes its enemy, rapidly smearing her with saliva and often biting her.

PARTHENOGENESIS

During November and December a number of experiments were conducted in the laboratory to determine whether parthenogenesis takes place. Unfertilized females were obtained from separate cocoons and were allowed to oviposit in small caterpillars, which they readily did. Males emerged from all the cocoons of *A. militaris* originating from these caterpillars, clearly showing that this species is parthenogenetic and indicating that unfertilized females give rise to a generation of males.

FEEDING EXPERIMENTS AND LONGEVITY

Adults which emerged on August 14 were confined in a lantern-globe cage in which grass was growing. They were fed on a mixture of honey and water, this being sprayed in minute droplets on the grass and walls of the cage. The adults were of both sexes and were kept alive for some time, the last one dying on September 1.

One female used in oviposition experiments was kept alive for eight days in a test tube, being fed honey, and another under the same conditions lived for seven days.

On November 6 and 7 a large number of newly emerged males were confined and fed in two lantern-globe cages indoors, as described above. These males were not allowed to copulate, and many lived until the first of December, the last dying on December 9 and 10.

WINTERING FORMS

All attempts at this station (La Fayette, Ind.) to winter this parasite under various conditions while in the cocoon have been unsuccessful. Mr. G. G. Ainslie, stationed at Nashville, Tenn., found this year (1915) that the army worm passed the winter there as young larvæ and, further, that specimens under observation were parasitized in the fall, for the parasites completed their growth and emerged this spring. Again, according to Gibson (1, p. 27), the army worm winters in Canada as

young larvæ beneath tufts of grass. Considering the data at hand, the theory is advanced that in the North the parasites winter as partly developed forms in immature larvæ, while in the South they no doubt also winter while in the cocoon.

ORIGIN AND FUNCTION OF THE CAUDAL VESICLE

The following is a summary of the results of the studies of Weissenberg and Seurat, together with the observations made by the writer, on the origin and function of the caudal vesicle, obtained mainly from experiments with hymenopterous endoparasites.

As Seurat's (7) and Weissenberg's (9) papers both deal with *A. glomeratus*, the caudal vesicle of which originates and functions identically as does that of *A. militaris*, the results of their studies are applicable to *A. militaris*. Weissenberg's paper, being the more exhaustive and, in addition, containing studies of the larva of this parasite in comparison with others less highly specialized, is used as a basis for this summary.

Observing the beginning of growth and the subsequent expansion of the caudal vesicle, the writer supposed that the entire proctodeum evaginated and turned inside out, but the careful histological studies of *A. glomeratus* by Weissenberg show that only a portion of the proctodeum through rapid growth becomes specialized to form the vesicle, while the remainder becomes temporarily atrophied. According to Weissenberg, the vesicle is formed by the rapid growth and elongation of the cells of the proctodeum which form the posterior end of the plug at the posterior end of the stomach, together with those adjacent cells at the anterior end of the proctodeum which surround the opening of the larval Malpighian tubules and extend posteriorly a short distance to the rudiments of the adult Malpighian tubules. The mass of elongated cells thus formed grows out through the anal opening of the embryo, and immediately following hatching these elongated cells are compressed lengthwise, so that their long axis becomes their short one, resulting in broad, flat cells joined edge to edge to form the thin wall of the caudal vesicle. During the rapid growth of these cells in the pyloric region the remainder of the proctodeum becomes atrophied and stays so until the caudal vesicle is drawn in. At this time parts specialized for endoparasitic life are reduced, and the atrophied parts grow rapidly, the whole approaching the normal proctodeal development of a free-living hymenopterous larva, previous to pupation.

Weissenberg next compares the origin and cellular structure of the caudal vesicle of *A. glomeratus* with that of the caudal appendage of the endoparasitic larval form of an undetermined species of Macrocentrus, and shows them to be homologous. In *Macrocentrus* sp., however, the cells always remain as a mass of long, slender cells protruding through the anal opening, a vesicle never being formed. The early stage of the

species of Macrocentrus studied was equipped with a tracheal system, while the corresponding stage of *A. glomeratus* was not. The conclusion is drawn that the vesicle functions as a blood gill in *A. glomeratus*, since all the blood necessarily pours through this vesicle, bathing its walls, while in *Macrocentrus* sp., which possesses a tracheal system, such an adaptation is not necessary.

An unknown species of the genus Limneria, parasitic on *Plutella cruciferarum* Zell., is next introduced for comparison by Weissenberg. In this parasite the portion of the proctodeum homologous with those of the two preceding larvæ discussed is not so well developed, for while pseudopod-like structures extend into the anal lumen, they do not protrude through the anal opening, which, however, is nevertheless very large. In this species it is clearly shown that the cells of these pseudopod-like structures completely correspond histologically with those of the larval Malpighian tubules. In a similar manner these specialized portions of the proctodeum of the two species last discussed are reduced and the portions retarded grow rapidly, approaching the normal proctodeal development of free-living larvæ before pupating, the normal proctodeal development of *Hemiteles fulvipes*, an ectoparasite of *A. glomeratus*, being used in comparison to illustrate this.

In the last analysis it is seen that the cells of these proctodeal appendages of the three endoparasitic larvæ considered are histologically allied with the cells of the larval Malpighian vessels, and with this in mind Weissenberg brings out clearly the idea that these proctodeal organs have also an excretory function and credits Kulagin (4, 5) with first suggesting this from results obtained from his injection experiments. Weissenberg further thinks that the excretory apparatus has undergone a superficial enlargement, owing to the active metabolism characteristic of this group, and that as excretory products in general are poisonous, it would seem natural to find here an adaptation by which they can be eliminated. His concluding argument is that in *A. glomeratus*, *Macrocentrus* sp., and *Limneria* sp. the development of the larval Malpighian vessels forms an ascending series, they being only rudimentary in *A. glomeratus* in comparison with the well-developed ones found in *Limneria* sp., while the proctodeal adaptations form a descending series, being most highly specialized and developed in *A. glomeratus* and only partly so in *Limneria* sp.

From the facts presented above and this study of *A. militaris*, the author concludes that the caudal vesicle is primarily an excretory organ and that the function of respiration is secondary. The following observations seem still further to strengthen this conclusion. The caudal vesicle functions from approximately the beginning of feeding to its close, and the portion of the first skin molted which covers the vesicle becomes greatly swollen in the second stage with a liquid content until finally it is ruptured. Further, the food of the larva is mainly the already digested solid parts

of the blood of the host, these being retained in the stomach during endoparasitic life, while the liquid parts, which are in excess, together with the by-products of anabolism and katabolism formed in the body of the rapidly developing larva, are eliminated by means of this enlarged adaptive excretory organ, which is bathed by the blood at each cycle. These by-products are doubtless eliminated from the body of the host, as are its own, by the Malpighian vessels. The caudal vesicle no doubt respires, this action taking place by osmosis, as is generally considered to be the case in endoparasites having a closed tracheal system. Whether respiration is more rapid through the walls of the caudal vesicle or whether they are especially adapted for it can not be positively stated, although Weissenberg, as stated previously, thinks that the vesicle functions as a blood gill. Again, that this portion of the body wall of the larva is apparently the thinnest and least chitinized is quite evident; therefore, it would not seem unreasonable to suppose that respiration takes place to a large degree through this area and that the air is carried mechanically throughout the body of the larva by the blood and is taken up from it to fill the closed tracheal system when it develops in the second instar.

Seurat's theory (7) that the essential function of the caudal vesicle is that of locomotion is no doubt incorrect, for careful observations of the movements of the larva show that the vesicle, because of its large size, is actually a hindrance to the larva in moving about in its host. Weissenberg (9) has also shown that the caudal vesicle is not homologous with the tail-like organs generally considered to be locomotor appendages which occur in various endoparasites, for both these organs are present in the larva of *Macrocentrus* sp. studied.

An additional point brought out by Weissenberg is that the caudal vesicle is an adaptation of the biophagous larva for its mode of life, for the necrophagous larva does not have it, and these adaptations arise from a biophagous mode of life in contrast with the necrophagous rather than from an endoparasitic life in contrast with an ectoparasitic life, as has been previously supposed.

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PLATE L

Apanteles militaris:

Fig. 1.—Diagrammatic drawing showing the embryo inclosed by the fused amniotic and serosal envelopes. *as*, Fused envelopes; *c*, chorion; *cv*, caudal vesicle; *h*, head.

Fig. 2.—Diagrammatic drawing showing the fused envelopes dividing into their two parts, the serosal cells being grouped at each pole. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

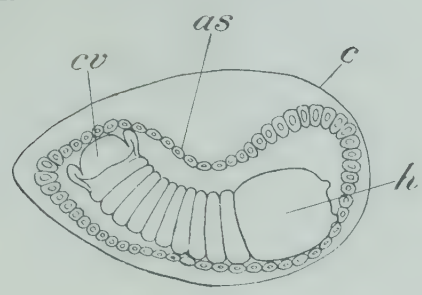
Fig. 3.—Diagrammatic drawing showing the egg ready to hatch, the serosal cells having become a loose mass and the embryo straightened out in the egg. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

Fig. 4.—Diagrammatic drawing of the larva during its first molt. *b*, Brain lobes; *cv*, caudal vesicle; *h*, head; *ht*, heart; *m*, molted skin; *mp*, Malpighian tubes; *o*, esophagus; *p*, pharynx; *sg*, silk glands; *st*, stomach; arrows indicate the blood cycle; *t*, rudimentary tube in the caudal vesicle connecting with the heart.

Fig. 5.—First instar. *cv*, Caudal vesicle.

Fig. 6.—Second instar. *cv*, Caudal vesicle.

Fig. 7.—Third instar, showing the position of the spiracles and the caudal vesicle withdrawn.



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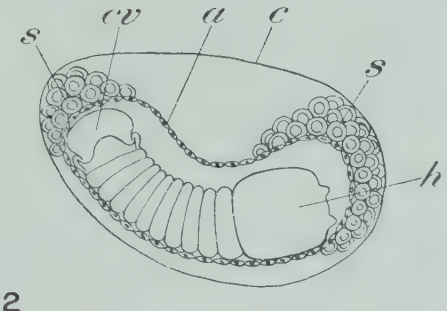

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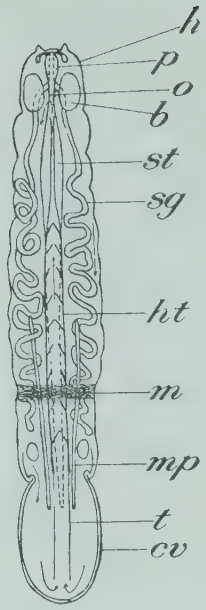

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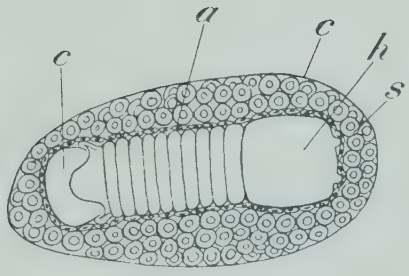

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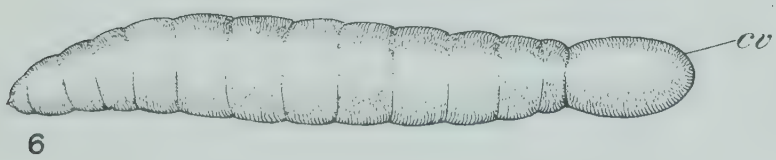

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RESPIRATION EXPERIMENTS WITH SWEET POTATOES

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INTRODUCTION

In 1882 Müller (7),[1] in the course of his classical researches on the accumulation of sugar in plant organs at low temperatures, observed that potatoes (*Solanum tuberosum*) which had been kept for a time at 0° C., and whose sugar content had in consequence been greatly increased, respired much more energetically than potatoes of lower sugar content. Even before the experiments of Müller, a number of analogous facts were known, all indicating that the respiratory energy of plants is a function of their carbohydrate content. Thus, isolated rootlets and seedlings deprived of their cotyledons show a rapid decrease in their respiration on account of the lack of plastic material normally furnished by the cotyledons (12). In etiolated seedlings the respiration curve rises at first as the food substances in the cotyledons or endosperm become available, and after passing a maximum falls gradually with the exhaustion of the food reserve (6, 11). The respiration of isolated leafy shoots kept in the dark sinks rapidly also, but if such shoots are exposed for a time to sunlight their respiration is considerably increased (1, 2). So also, if the carbohydrate content of etiolated leaves, shoots, or seedlings is increased by an immersion of the parts in sugar solutions, respiration is greatly stimulated, although Palladine attributes the increased respiration partly to the formation of active proteins produced under conditions of favorable carbohydrate nutrition (5, 8, 9).

Since the sugar content of sweet potatoes (*Ipomoea batatas*) changes greatly in storage, it appeared not unlikely in view of the foregoing facts that their respiratory activity would show corresponding changes at different seasons. The experiments described in the following pages were performed in order to ascertain whether any such correlation exists between the seasonal changes in the sugar content of sweet potatoes and their respiratory activity, and incidentally to determine if possible whether the monosaccharids or the disaccharids of the sweet potato furnish the chief material for respiration. The roots were taken from the lots stored for experimental purposes under the conditions described by the writers in a former paper (4). The details are given in connection with the descriptions of the individual experiments. The respiration

[1] Reference is made by number to "Literature cited," p. 517.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bk

Vol. V, No. 12
Dec. 20, 1915
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experiments were all carried out at 30° C. This temperature was chosen in order to study the respiration of the sweet potatoes under conditions similar to those to which the freshly dug roots are subjected during the curing process, which consists essentially in keeping them at a temperature in the neighborhood of 30° C. for about 10 days.

EXPERIMENTAL METHODS

The methods employed in the experiments require but little description. The sweet potatoes were placed in a large receptacle in an ordinary water-jacketed incubator, which was kept at a temperature of 30° C. A current of air having the same temperature and freed from carbon dioxid was drawn through the receptacle at the rate of 40 to 50 liters per hour. The carbon dioxid of respiration was collected in approximately one-half normal potassium-hydroxid solution, whose titre for pure potassium hydroxid had been determined. The absorption was effected by means of Reisette flasks. At the end of every 24-hour period the carbon dioxid in the Reisette flask was precipitated by means of an excess of barium chlorid, and the residual potassium hydroxid was determined by titration with normal or half-normal hydrochloric acid.

About 2 to 3 kgm. of sweet potatoes were used in each experiment. At the beginning of the experiment the sugar content was determined in a collateral sample of 3 to 4 kgm. from the same lot. At the end of each experiment all the sweet potatoes which had been used for that experiment were ground and sampled for determinations of sugar and moisture. The figures giving the sugar determinations are averages of five samples from each lot. The directly reducing sugar was calculated as glucose. The soluble carbohydrates yielding reducing sugar after inversion were calculated as cane sugar, which is the most abundant disaccharid present in the sweet potato. Jersey Big Stem sweet potatoes were used in all the experiments.

EXPERIMENTAL DATA

The results of all the experiments are collected in Table I. The percentages of total sugar (as glucose), cane sugar, and reducing sugar (as glucose) in the collateral sample taken at the beginning of each experiment, and in the experimental sweet potatoes at the end of the experiment, are given at the head of the table. These figures were in each case calculated for sweet potatoes of the water content of the collateral sample—i. e., the assumed original water content of the experimental sweet potatoes. The carbon-dioxid output is given in milligrams per kilogram per hour for each day. In the calculation the loss of weight of the sweet potatoes during the experiment was taken into consideration and was distributed uniformly over the period. At

the end of Table I is given the gain or loss of reducing sugar, calculated from the analytical data, and the glucose equivalent of the total carbon dioxid generated during each experiment, as actually determined. The percentages of reducing sugar in the sweet potatoes at the end of each experiment, without correction for changes in water content, were as follows: First experiment, 1.24 per cent; second, 1.22 per cent; third, 1.39 per cent; fourth, 0.91 per cent; fifth, 0.71 per cent; sixth, 0.67 per cent; seventh, 0.69 per cent. The experiments themselves will be described individually.

TABLE I.—*Composition and carbon-dioxid output of sweet potatoes at different times of the year*

Item.	Period.	Experiment 1, Oct. 21 to Nov. 5.	Experiment 2, Nov. 7 to Nov. 17.	Experiment 3, Dec. 9 to Dec. 19.	Experiment 4, Jan. 4 to Jan. 15.	Experiment 5, Mar. 26 to Apr. 5.	Experiment 6, Apr. 16 to Apr. 26.	Experiment 7, June 1 to June 11.
Total sugar (as glucose), per cent.	At beginning of experiment.	2.62	5.80	8.99	6.22	7.03	7.41	7.30
	At end of experiment.	5.38	5.08	8.42	5.82	7.45	7.40	7.20
Cane sugar, per cent.	At beginning of experiment.	1.60	3.41	6.58	4.63	5.82	6.17	6.08
	At end of experiment.	3.95	3.72	6.74	4.71	6.42	6.41	6.21
Reducing sugar, per cent.	At beginning of experiment.	.94	2.21	2.06	1.35	.90	.92	.90
	At end of experiment.	1.23	1.18	1.35	.87	.70	.67	.68
Daily rate of carbon-dioxid output, mgm. per kgm. per hour.	1st day	27.7	73.9	138.2	49.1	50.9	46.6	47.5
	2d day	24.9	82.1	144.9	56.0	44.9	49.4	42.4
	3d day	36.5	70.9	116.4	54.1	47.0	48.4	43.4
	4th day	35.7	60.0	101.8	48.4	46.8	46.4	46.2
	5th day	37.1	51.8	92.9	48.2	47.5	43.3	42.9
	6th day	31.8	45.9	90.4	44.0	47.0	42.0	43.1
	7th day	41.7	40.4	84.9	42.6	46.1	42.1	40.7
	8th day	34.3	39.1	83.3	40.7	46.5	39.3	42.2
	9th day	31.6	34.8	76.7	39.8	44.9	40.8	41.0
	10th day	29.8	32.8	79.9	38.9	41.1	39.3	44.1
	11th day	28.8	.....	.....	41.4	.....	.....	.....
	12th day	28.5	.....	.....	.....	.....	.....	.....
	13th day	24.9	.....	.....	.....	.....	.....	.....
	14th day	31.7	.....	.....	.....	.....	.....	.....
	15th day	29.9	.....	.....	.....	.....	.....	.....
Increment in reducing sugar calculated from the analytical data, gm.	.....	9.77	−31.69	−17.35	−10.03	− 3.40	− 5.14	− 2.50
Loss of reducing sugar equivalent to the carbon dioxid evolved, gm.	.....	27.45	25.85	35.18	15.80	11.73	13.41	7.33

EXPERIMENT 1.—In this experiment 3,576.5 gm. of sweet potatoes were used. These were dug on October 20. The experiment was begun on the following day and continued until November 5. During that period the cane-sugar content rose from 1.60 to 3.95 per cent and the invert-sugar content from 0.94 to 1.23 per cent. The respiration rose somewhat during the first half of the period and then fell to a nearly uniform rate of approximately 28 mgm. per kilogram per hour. The rise at first, which was observed in nearly all the other experiments also, may in part be attributed to the rise of the temperature of

the sweet potatoes when they were put into the incubator. Although there is a marked increase in both cane sugar and reducing sugar in the sweet potatoes, there is no evident general rise in the respiratory activity corresponding to the increase in the sugar content. During the course of the experiment the equivalent of 27.45 gm. of glucose was given off by the sweet potatoes as carbon dioxid, yet during this period 9.77 gm. of reducing sugar accumulated in them. The loss of weight of the sweet potatoes was 77 gm.

EXPERIMENT 2.—The sweet potatoes used in the second experiment were of the same lot as those of the first, but they had stood in the laboratory at a temperature of about 20° C. until November 7. The weight of the roots used for the experiment was 3,029.8 gm. The loss of weight was 138.8 gm. The percentage of cane sugar rose slightly, but the reducing sugar fell from 2.21 to 1.18 per cent. The respiration was high at first and fell gradually, apparently with the decreasing percentage of reducing sugar. It is clear that if in this case the lowering of the respiratory activity is due to the decrease of sugar, the effect must be wholly attributed to the change in the invert-sugar content, since the cane sugar, so far as may be judged from the analysis of the collateral sample, remained stationary or even rose slightly. The changes in the quantity of reducing sugar in these sweet potatoes are of special interest, for here the quantity of reducing sugar lost, according to calculations based on the analytical data, is greater than that lost through respiration as calculated from the quantity of carbon dioxid evolved. It seems, therefore, that a portion of the reducing sugar was used for other processes than respiration, possibly for the production of cane sugar.

EXPERIMENT 3.—The sweet potatoes used in the third experiment had been subjected to the regular curing process and had thereafter been kept in cold storage at a temperature of 6° to 7° C. from November 8 to December 9. The roots used in the experiment weighed 2,207.2 gm., and their loss of weight was 184.2 gm. As a result of the exposure to low temperature, the sugar content of these sweet potatoes was higher than of those used in any of the other experiments. The respiration of these chilled roots was also very high, but sank rapidly toward the end of the experiment. The quantity of reducing sugar equivalent to the carbon dioxid evolved in respiration was greater than the apparent decrease calculated from the analytical data.

EXPERIMENTS 4, 5, 6, AND 7.—The remaining experiments all present a certain uniformity and may be described together. The sweet potatoes used in these experiments were cured in the usual manner and were thereafter stored at a temperature of 12° to 15° C., until the dates on which they were used. The weights of the sweet potatoes used in the different experiments were 1,984, 1,577.5, 1,898.5, and 1,054.5 gm., respectively. The corresponding losses were 143, 56.5, 59.3, and 40.8

gm. The sugar content of these lots was remarkably uniform. Only the lot used in the fourth experiment was lower in cane sugar and higher in reducing sugar than the rest. In spite of this difference, the respiration in all cases was practically the same, beginning in the neighborhood of 50 mgm. per kilogram per hour and falling to about 40 mgm. toward the end of the experiments. In all cases the glucose equivalent of the carbon dioxid generated was higher than the loss of reducing sugar calculated from the analytical data.

DISCUSSION OF RESULTS

A comparison of the sugar content of the sweet potatoes in the different experiments with the respiration of the roots shows that no general correlation is evident between the total sugar content and the respiratory activity. It is true, indeed, that the roots having the highest sugar content (third experiment) also had the highest respiration, but these sweet potatoes had been subjected to low temperature for a month, and it is likely that such treatment induces other changes than those indicated by the carbohydrate transformations, for sweet potatoes thus treated become subject to the attacks of certain fungi which ordinarily do not readily invade the tissues. Moreover, it appears from experiments of Palladine (10) that, with a plentiful supply of carbohydrates present, plant organs which have been exposed for a time to low temperature respire more energetically when brought into a high temperature than those which have been continually kept at the higher temperature. Furthermore, the carbon-dioxid production in the third experiment fell off rapidly until it was no greater than that at the beginning of the second experiment, but the total sugar content of the sweet potatoes in the third experiment remained at all times much higher than that of the roots in the second experiment. The other experiments also show no correlation between the total sugar content of the sweet potato and the respiratory activity. Thus, the roots in the second experiment were low in total sugar, but had a high respiration, while those in the fifth, sixth, and seventh experiments had a comparatively high sugar content and low respiration. It is possible that irregularities in the size and shape of the sweet potatoes might account for differences in respiratory activity, but these sources of error were avoided as far as possible by the selection of fairly uniform roots. It is therefore unlikely that great differences in respiratory activity can be attributed to these factors.

While there appears to be no evident correlation between the total sugar content and the respiratory activity, the case is different when the reducing sugar alone is considered. Here there is evidence of a general parallelism, which, however, is easily obscured by other factors. This correlation is perhaps most clearly brought out by the gradual fall of the respiration, with the disappearance of the reducing sugar in the indi-

vidual experiments. The first experiment, however, is in marked contrast to the others in this respect, for, although the sugar content of these sweet potatoes rose from 0.94 to 1.23 per cent, there was no corresponding rise in the respiration. The parallelism between the respiration and the sugar content is less marked when the different experiments are compared. Thus, the roots in the second experiment contained approximately the same percentage of reducing sugar as those in the third, yet the respiration was much lower in the second. This fact, as has been pointed out, may probably be ascribed to the treatment to which the sweet potatoes had been subjected before the experiment. It is evident on the whole that the respiratory activity of the sweet potatoes is as greatly influenced by seasonal changes and environmental factors to which they have been exposed as by the sugar content. It is clear, of course, that with the exhaustion of the carbohydrates immediately utilized in respiration, the rate of respiration will fall, as in the case of seedlings grown continually in the dark, but it seems that an increase of the available carbohydrate supply does not necessarily entail a continued increase in the respiratory activity. That there is sufficient sugar present in sweet potatoes, as well as in plant organs generally, to support a more active respiration than usually takes place, is shown by the increased respiration as a result of wounding. Table II gives the carbon-dioxid output per kilogram per hour of two lots of sweet potatoes for a short period before and after they were split lengthwise.

TABLE II.—*Carbon-dioxid output in milligrams per kilogram per hour of two lots of sweet potatoes for a short period before and after being split lengthwise*

Before roots were split.			After roots were split.		
Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.	Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.
	Mgm.	*Mgm.*		*Mgm.*	*Mgm.*
1	4.4	42.7	7	9.3	60.0
2	4.1	39.2	8	6.9	50.8
3	4.7	36.3	9	7.2	52.7
4	5.4	35.4	10	7.2	70.7(?)
5	5.7	32.8	11	7.4	56.4
6	5.6	29.8	12	7.3	54.5
			13	7.6	52.5

The great increase in respiration after the sixth day, when the roots were split, shows that there was sufficient sugar present to support a more energetic respiration than that which took place in the whole roots, but that other limiting factors than the sugar supply determined the rate of respiration.

In the consideration of the question of the relative availability of the monosaccharids and the disaccharids as sources of material for respira-

tion, a certain allowance should perhaps be made for the nonconformity of samples, since the sugar content of the sweet potatoes at the beginning of each experiment was necessarily determined in collateral samples. Nevertheless, two facts appear evident. During the course of the experiments there was no diminution, but, on the contrary, an increase, in the quantity of cane sugar present in the sweet potatoes, while there was a marked decrease in the reducing sugar in all the experiments except the first.

The rise in the cane-sugar content of the sweet potatoes is most marked in the first experiment, but in this case the rapid change is simply an example of the generally observed manifestation that the sugar content of sweet potatoes is low while they are in the ground and rises rapidly immediately after they have been dug. In all the other experiments, although the increase is small (from 0.08 to 0.6 per cent), the differences all point in one direction. It seems clear, therefore, that there was at any rate no decrease in the cane-sugar content of the sweet potatoes during the course of the experiments.

This fact indicates that at 30° C. the cane sugar is reformed as rapidly as it is used for respiration or that it does not function in the respiratory processes, at least while other carbohydrates are present in abundance. Which of these possibilities occurs can not with certainty be determined from the data. A number of relative facts, however, seem to point to a rather high degree of stability of the cane sugar in the sweet potato, in so far as the processes of respiration are concerned. It has been found as the result of many analyses that at low temperatures (5° C.) there is an extensive accumulation of cane sugar in the sweet potato and that this increase of sugar takes place at the expense of the starch, which disappears correspondingly. At higher temperatures (15° to 20° C.) the accumulation of cane sugar is much less extensive and, in fact, does not proceed beyond a certain maximum, which, during the season's storage, is reached in March or early April. After the period of sugar formation the starch content of the sweet potatoes remains fairly constant, for the quantity of starch which disappears in respiration compared with the quantity used in the formation of sugar is so small that in view of individual differences among sweet potatoes and the errors of manipulation it has not been possible to determine the changes in starch content in connection with respiration in experiments carried on for short periods of time.

These facts seem to indicate that at higher temperatures the production of cane sugar is depressed. We should therefore expect that if sweet potatoes which have been stored at 15° to 20° C. until the cane-sugar content has attained an equilibrium (March to April) are subjected to a temperature of 30°, the production of cane sugar would be still further retarded or even inhibited. At the same time the rate of respiration is accelerated.

If no more cane sugar is formed and its utilization is hastened, we should expect a reduction in the quantity of cane sugar, at least in the experiments at the end of the season, if that substance is used in respiration. Such a reduction, however, occurs neither at the end of the season nor at any other time. It appears not unlikely, therefore, that the cane sugar in the sweet potato is relatively stable, with respect to the respiratory processes.

Although there was no diminution of cane sugar in the sweet potatoes used in these experiments, there was a marked decrease in the reducing sugar in all cases except the first. The first experiment, in which freshly dug roots were used, is exceptional for the reason mentioned above. It shows that in freshly dug roots the processes of sugar formation are so rapid that even at 30° C. sugar is formed faster than it is used in respiration. In this instance an amount of carbon dioxid equivalent to 27.45 gm. of glucose was evolved during the experiment, and in addition to this there was an increment of 9.77 gm. of reducing sugar, as calculated from the percentages present in the sweet potatoes at the beginning and at the end of the experiment. In all the other experiments there was a decrease of reducing sugar—i. e., the quantity of reducing sugar which had accumulated while the sweet potatoes were stored at low temperatures was diminished when the roots were subsequently exposed to a higher temperature. It is reasonable to infer that the sugar was utilized in respiration, but it will be observed that in all but the first and second experiments the loss of reducing sugar calculated from the percentages at the beginning and at the end of the experiments accounts only for a portion of the sugar equivalent to the quantity of carbon dioxid evolved. The deficiency is no doubt made up by the transformation of starch, for, as Deleano (3) found in the case of grape leaves cut from the vines, the starch functions readily in the respiratory processes. In the sweet potato the starch appears to be even more readily available than the cane sugar. In the second experiment, where the invert-sugar content was high at the beginning of the experiment, a synthesis of other carbohydrates may perhaps be assumed.

CONCLUSIONS

The experiments described in this paper seem to indicate that there is no general correlation between the total sugar content of the sweet potato and its respiratory activity. A simultaneous decrease in the reducing-sugar content and the respiratory activity of given lots of roots indicates a correlation between reducing-sugar content and respiration, but seasonal changes and environmental conditions to which the sweet potatoes have been previously subjected tend to obscure any such correlation in different lots. Experiments with wounded roots indicate that the sugar content is not the limiting factor in the respiration of the sweet potato. The reducing sugars are the immediate source of respira-

tory material. The cane sugar is relatively stable in the sweet potato, and when once formed it does not appear to be readily utilized in the process of respiration, while starch and other carbohydrates are present in abundance.

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CHERRY AND HAWTHORN SAWFLY LEAF MINER

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INTRODUCTION

The existence in the State of New York of a leaf miner attacking cherry (*Prunus* spp.) foliage was brought to the attention of the Experiment Station by the receipt of affected foliage during the latter part of June, 1910. An examination of the orchard from which the material had been collected showed that more or less of the leaves on nearly all of the trees of a variety known as English Morello had shriveled and died, while here and there were others with well-defined light-colored areas or blisters, revealing a loss of chlorophyll. Siftings of earth from beneath the trees showed that the causal agent was the larva of a species of sawfly. A number of these were carried through successive stages of development to the following year, when adults were obtained. Some specimens were forwarded to Dr. A. D. MacGillivray, formerly of Cornell University, who reported that the insect represented a new species, the type of a new genus, and should be recorded as *Profenusa collaris*. The information was also given that the creature had been reared from the hawthorn (*Crataegus* spp.).

HOST PLANTS OF SAWFLY LEAF MINER

According to present knowledge, the host plants of the sawfly leaf miner are the cherry and the hawthorn. Of the cherries, it has so far largely confined its attacks to the English Morello variety. It is not commonly observed with the Montmorency or Early Richmond, which would indicate that its presence on these varieties is accidental and occurs when they are grown in proximity to the English Morello. The susceptibility of one fruit and the apparent unattractiveness or resistance to the insect of the other fruits is a curious fact, since all are cultivated varieties of the same cherry, *Prunus cerasus*, and plantings of each kind, growing side by side, may be frequently observed in this State. The two sorts, Montmorency and English Morello, represent groups of cherries which vary more or less in both tree and fruit but have a constant difference only in a single character—the juice in the fruits of one is colorless; in the other it is red. This sharp discrimination on the part of the sawfly leaf miner seems all the more anomalous when considered in the light of its extreme partiality to the foliage of certain hawthorns which are only remotely related to the cherry.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 12
Dec. 20, 1915
N. Y. (Geneva)—4

In its attacks on hawthorns the leaf miner tunnels the foliage in the same manner as that of the cherry. During the course of our studies it has been very evident that the pest is more destructive to certain species of Crataegus than it is to the English Morello cherry. As has been rarely observed in the case of the latter plant, one may find as many as five larvæ mining a single leaf. With hawthorns having a relatively small and narrow leaf, as *C. geneseensis*, there may be an entire destruction of the pulpy tissue, in which event all that remains of the affected leaf is the epidermis, which dries up and ultimately falls to the ground. At the height of an attack, which occurs when the larvæ are reaching maturity, hawthorns which are much infested take on a brownish cast and appear as if struck by a blight or swept by fire. In decorative plantings the destructive work of the insect may assume such a character that the attractiveness of certain species of hawthorns as ornamental shrubs is seriously marred.

About Geneva the sawfly leaf miner is most common in the foliage of an unidentified hawthorn belonging to the Medioximae group, while such species as *C. pedicellata* and *C. punctata*, growing in the immediate vicinity of the former, have so far shown little or no injury and are generally exempt from attack. Dr. C. S. Sargent, Director of the Arnold Arboretum, writes that the insect has become established in the plantings of *Crataegus* spp. and that it is especially destructive to hawthorns of the *crus-galli* group and to *C. nitida*, *C. rotundifolia*, *C. pruinosa*, and other species. Similar conditions exist at the New York Botanical Garden and, as elsewhere, certain species of Crataegus are quite badly infested, while a few species have so far been free from attack.

In the public parks at Rochester, N. Y., notably Genesee Park, the insect has in recent years become a serious pest. Hawthorns representing a wide range of species and grown in extensive numbers feature prominently in certain landscape plantings. In these the sawfly leaf miner has become established, and its destructiveness may be readily observed during May and June. Some haws have been seriously affected, while others have been exempt from injury. Here, again, various hawthorns of the *crus-galli* group have proved to be very susceptible to the pest, and certain species of other groups have shown considerable injury.

DISTRIBUTION OF SAWFLY LEAF MINER.

As a cherry pest the sawfly leaf miner is definitely known to occur in injurious numbers in orchards of English Morello cherry about Geneva in western New York and about Germantown, which is located in the Hudson Valley. It has been reported to the Station as occurring about Schenectady, but the statement of its presence in that locality has not been verified. In view of its occurrence in two communities which are widely separated, it would seem reasonable to suppose that the pest exists in

other localities where sour cherries are extensively grown. However, a careful survey by the orchard and nursery inspectors of the Department of Agriculture in all of the leading fruit-growing counties of the State has failed to find any evidences of the work of the insect except in the foregoing localities. A study of available literature indicates that the insect is not known to occur as a cherry pest outside the State of New York.

As a depredator of hawthorns the sawfly leaf miner has a wider range of distribution. It is known, as already indicated, as a serious pest of hawthorns growing about Boston, Mass., and it is common on various species of Crataegus growing in the vicinity of New York City, Rochester, Ithaca, Geneva, and Skaneateles, all of which are located in the State of New York.

APPEARANCE OF THE INJURY

As implied by its common name, the insect is a leaf-mining species and its work is very characteristic. The injury is first indicated by a small, thin, sinuous channel which finally swells out into a large blister-like area of a light-brown color, resembling that of dead leaf tissues. The attack by the larva of the sawfly leaf miner begins on the edge of the leaf toward the stem and continues along one side toward the leaf apex, the tunnel increasing in dimensions with the growth in size and the progress of the insect. Upon reaching the tip of the leaf the grub reverses its course and works backward toward the stem, consuming the remainder of the pulpy tissues between the main rib and the margin of the leaf. As a result, the parenchyma, or soft cellular tissue, is eaten, leaving the epidermis, which turns brown and forms a large blister. These blisters are very conspicuous on the upper surfaces of the leaves. Oftentimes the whole leaf is mined, but usually with most of the foliage only from one-quarter to one-half of the whole area of a leaf is destroyed. (Pl. LI, fig. 1.) Only the leaves that first unfold are subject to attack, and during some seasons hardly any of these escape the insect's depredations. The principal damage occurs during the last week of May and the early part of June, or about one month before the harvesting of the fruit. With the disappearance of the larvæ the leaves most seriously affected shrivel, die, and finally drop to the ground, causing defoliation, which varies in importance according to the extent of infestation and the influence of seasonal conditions on the rate of growth.

The actual effect of the work of the insect upon the crop is not easily measured and during most years is perhaps not of serious extent. However, as previously indicated, the destructive power of the pest is mainly exercised on the leaves that unfold with the bursting of the buds. In years of slight precipitation and when new growth is of small extent and of slow development the plant is dependent on such foliage as it carries at the time, and any extended injury to it must result in a set-

back, with correspondingly ill effects on the maturing crop of fruit. In years when the production of new growth is more rapid the damage caused by the sawfly leaf miner is of much less importance, as the large leaf surface under the circumstances is sufficient for the needs of the plant, and the loss of affected foliage does not result in an important reduction in leaf area.

The hawthorns are more subject to severe attacks than the cherry, and during some seasons plants may be observed on which there is hardly a leaf that does not show injury. Notwithstanding the partiality of the sawfly leaf miner for this plant, hawthorns seem able to withstand considerable destruction of foliage without marked external evidences of the weakening of the tree. As shown in Plate LI, figure 2, the attractiveness of the plants as ornamental shrubs may be seriously marred.

DESCRIPTION OF LIFE STAGES OF SAWFLY LEAF MINER

EGG

The egg is elliptical in shape, but is not entirely symmetrical in its outline, as one side shows a greater curvature than the other. It is, when removed from surrounding plant tissues, circular in cross section, but in its normal position in the leaf structure it is much flattened, owing to pressure. The chorion is a thin, white, shining, flexible membrane. The measurements of eggs when not compressed are: Length, 0.5 to 0.7 mm.; diameter, 0.28 to 0.36 mm.

LARVA

To determine the number of instars, the mines were carefully examined for all insect remains, when the head molts were collected and measured as to width. The body remnants from some of the molts in first larval instars were occasionally missing, having probably been eaten, but in very few cases were the head structures not in good condition for examination. The width of the head is fairly constant for the first larval instar, but in the more advanced stages there is considerable variation. On the basis of head measurements it appears that the larva normally molts five times in its mine. It finally enters the ground and molts again in transforming to a pupa.

The first five instars have the same general form and differ one from the other principally in size. The body is broadest at the first and second thoracic segments and gradually tapers toward the rear. The thoracic legs are short and conical and are composed of five segments, which include the thick basal and the small hooked terminal structures. All the abdominal segments except the last bear short rounded prolegs on the ventral side. The head is horizontal in the early stages, but slopes downward slightly in later instars. It is broad and flat, rounded on the sides, and obtuse in front. On the dorsal side it bears four longitudinal sutures. The outer pair run back from the ends of the clypeus and divide the head into three almost equal sections. The inner pair extend halfway across the middle section, dividing it into three equal areas. The eyes are wanting. The antennæ are very short and are apparently composed of three segments. The maxillary palpi are large and protrude from beneath the head. The labial palpi are very small. The mandibles are short and thick, deeply hollowed on the inner side, and do not protrude beyond the end of the broadly notched labrum.

The technical description of each of the larval stages follows:

FIRST INSTAR.—Body translucent, white, shining; only slightly wrinkled, and with a green streak, due to alimentary tract, showing plainly in the abdominal segments. Prolegs appear as only slight elevations.

Head is slightly brownish, being of dark color on the outer and posterior edges; mouth parts are reddish brown. The ventral side of the first thoracic segment has a pair of brownish gray marks, shaped roughly like a T, with the cross bar running longitudinally and the perpendicular reaching outward to a point just in front of the leg. A semicircular line of the same color occurs in front of the anus and is interrupted on the median line.

Newly hatched larvæ are about 1.2 mm. in length, and after feeding, the body grows, reaching a length of 2.3 mm. Width of head, 0.36 to 0.42 mm.; average, 0.39 mm.

SECOND INSTAR.—All markings of body are more extensive than in preceding stage. Dorsal side with some specimens has a broad, faint, brownish gray, transverse band on the first thoracic and two spots on the second thoracic segment. The pair of marks on ventral side of first thoracic segment are shaped more like inverted V's, and between them there is a large longitudinal band. The second and third segments have median oval spots. Each proleg is marked by a narrow crescent on the anterior side. A semicircular mark on the last segment extends over half a circle and is not interrupted on the median line.

Length, 2.6 to 3 mm. Width of head, 0.48 to 0.55 mm.; average, 0.52 mm.

THIRD INSTAR.—All markings are the same as in preceding stage, but are much fainter. Prolegs are more prominent; those on the first and penultimate abdominal segments are small.

Length, 3.2 to 4.3 mm. Width of head, 0.63 to 0.73 mm.; average, 0.67 mm.

FOURTH INSTAR.—The characteristic markings in preceding stages practically disappear in this instar. A ring of several rows of minute papillæ surrounds the anus. These probably exist in the earlier instars and escape detection because of their small size.

Length, 4.5 to 7.2 mm. Width of head, 0.8 to 0.9 mm.; average, 0.85 mm.

FIFTH INSTAR.—This is similar to fourth instar. There are no distinct color markings.

Length, 6.5 to 7.5 mm. Width of head, 0.92 to 1.07 mm.; average, 1 mm.

SIXTH INSTAR.—The body does not differ from that of preceding stage. The head assumes a vertical position. The four sutures on the dorsal side are very faint. The clypeus and labrum are shorter than in fifth instar. The mandibles protrude prominently and do not meet at the ends. The labium and maxillæ project from beneath the head to beyond the tips of the mandibles.

Length is same as in fifth instar or may be a trifle shorter. Width of head, 0.90 to 1.05 mm.; average, 1 mm.

PUPA

Until color of adult begins to show, the pupa is white in all portions except the eyes, which are reddish. Length about 5 mm.

ADULT

"Body [of female] black, with the clypeus, labrum, malar space, the mandibles, the first segment of the antennæ, the tegulæ, a narrow margin to the pronotum, and the legs, for the most part, whitish. The prothorax, except the parts named, the cephalic part of the mesopleuræ, and the pectus, rufous; the posterior femora more or less shaded with fuscous; the head smooth with antennal furrows interrupted on the middle of the face; the furrows surrounding the postocellar area deep and distinct, the vertical furrows not reaching the occiput; the median ocellus placed on a flat depression; a pit above the antennal socket; the median fovea minute but dis-

tinct; the clypeus truncate; the first and second antennal segments subequal, the third segment subequal to one and two together and longer than four; the saw-guides with the dorsal and ventral margins converging and the apex bluntly pointed; the male differs in having the rufous part of the thorax inclined to whitish and extending over the entire pleuræ, the venter of the abdomen and a broad band on the lateral part of the dorsal aspect, broader behind, sometimes fused on the meson, whitish; the posterior femora not fuscous. Length 3 to 4 mm."[1]

LIFE HISTORY AND HABITS OF SAWFLY LEAF MINER

EMERGENCE OF ADULTS

From puparia obtained on April 18, 1913, by sifting earth from beneath cherry trees, two male and seven female sawfly leaf miners made their appearance during a period extending from April 28 to May 2. On May 6 six males and six females were obtained in a cherry orchard, and only one of the flies was obtained in cages intended to trap the insects as they emerged from the ground. On May 7 five males and seven females were caught in breeding cages, and at this date the insects were present in large numbers on the trees. The insects continued to appear in the cages, a few each day, until May 19, which for 1913 was the latest date for the emergence of the flies for that year. Observations for several seasons show that the flies make their appearance when the first leaf clusters are unfolding and the cluster buds are beginning to open.

EARLY HABITS

At the time of their emergence from the ground the sawfly leaf miners are fully colored and are very active creatures. They are apparently very susceptible to temperature conditions. If disturbed on cold days, they drop suddenly from the foliage, attempting to fly while in midair. Failing in this effort, they drop to the ground and crawl to some elevated object, on which they renew their attempts to seek flight.

They copulate within less than a day after their appearance from the soil. In this act the male approaches the female backward, so that the tips of their abdomens come in contact while their heads are opposed to each other. Then the male reaches back with the hind legs and grasps the female over the back of her body, placing at the same time the tip of his abdomen under that of the female and inserting the penis under the flap at the base of the ovipositor. The outer flaps of the male genitalia are pressed closely against the under side of the female's body. The whole process is a matter of one to three minutes. One pair contained in an observation jar copulated three times within a space of half an hour.

OVIPOSITION

The females are apparently ready to oviposit soon after they make their escape from the ground. One specimen was dissected about 17 hours after its appearance, and in the ovaries and oviducts there were

[1] MacGillivray, A. D. New genera and species of sawflies. *In* Canad. Ent., v. 46, no. 10, p. 364–365. 1914.

counted 15 fully developed eggs. Another that had been out for two days began to deposit eggs immediately when cherry leaves were introduced into its cage. In the orchard eggs were first found during the year 1913 on May 7; in that season adults were first observed on May 6, although the insects may have been present on the trees for a day or two before and escaped detection. During the first days of the oviposition period one or sometimes two leaves in a cluster may show the presence of eggs. The females seem to manifest a preference for leaves which are first to appear and which are partly folded. The process of oviposition requires only about a minute. Details of this operation proved difficult to determine because of the extreme shyness of the females, which fly quickly on the approach of any object.

The lower surface of the egg lies in contact with the lower epidermis, which has been cut free from the other tissues of the leaf so as to form a small blister-like cavity or pocket. The egg is usually within 1 or 2 mm. from the edge of the leaf; rarely on the extreme edge or more than 3 mm. from the margin. On the upper side at the edge of the cavity there is usually a stoma, through which the ovipositor is probably thrust. An examination of 91 eggs at random shows that they are more often deposited near the base of the leaf than the tip. About 70 per cent of the eggs were in the area of the leaf from one-eighth to one-third the distance from the base, 20 per cent near the middle, and about 10 per cent occurred in the portion of the leaf toward the tip. From 1 to 5 eggs were observed on a single leaf, and the average for all observations was 2.3 eggs per leaf.

HATCHING AND LARVAL ACTIVITIES

During 1913 young larvæ were first observed on May 24 as the trees were coming into full bloom, but judging from the sizes of some of the mines it was evident that a few eggs had hatched one or two days earlier. By May 27 the hatching period was practically completed. In the field it proved difficult to determine the period of incubation, but eggs deposited on cherry leaves in the insectary hatched in eight days from date of oviposition. Under normal conditions incubation would probably extend over a larger number of days.

Upon hatching, the young larva works its way through the tissue of the leaf until it reaches the upper epidermis. It usually mines toward the distal end of the leaf, generally keeping close to the edge and feeding with the ventral side in contact with the upper epidermis. When the tip of the leaf has been reached the creature reverses its course, proceeding along the area adjoining the midrib; or if there is no interference by another larva it may cross over the main rib and tunnel back along the edge of the opposite half of the leaf.

The mine, as viewed from above, during its first stages of development is rather dark brown in color, which is accounted for in part by frass along the edges of the roof of the tunnel. As the affected area increases

in size, especially in its breadth, the mine becomes light brown, while the edges incline to a darker shade. Observed from beneath, the only visible indication of the initial activities of the insect is a small oval spot, which marks the original cavity constructed by the adult for the reception of the egg, and this contains in addition to the shriveled egg membrane accumulations of frass from the early feeding operations of the larva. Later, the underside of the tunnel also becomes brown, with the exposed epidermis wrinkled, but, in general, the destructive work of the insect is not so apparent on the lower as on the upper surface of the leaf.

There is a fairly definite relationship between the size of the mine and the age of the larva with respect to the different instars. In general, mines under 5 mm. long and 2 mm. at their greatest width contain larvæ in the first instar; mines that are 5 by 2 mm. to 12 by 4 mm. contain larvæ in the second instar; mines that are 8 by 5 mm. to 8 by 6 mm. contain larvæ of the third instar; mines that are 18 by 6 mm. to 28 by 8 mm. contain larvæ of the fourth instar; and mines of greater dimensions than the foregoing are occupied by larvæ of the fifth instar.

PUPATION

Upon reaching maturity the larvæ make a hole in the tissues forming the mine, usually the upper epidermis, which forms the roof. From the opening they make their escape to the edge of the leaf, when they drop to the ground. During 1912 the larvæ began to leave the foliage on June 7, and by June 10 it was estimated that 50 per cent of the insects had abandoned their mines. On June 18 it was difficult to find a specimen on the tree, while June 22 was the latest date that any of the insects were seen on the leaves. Upon reaching the ground they bury themselves several inches deep in the soil and construct an earthen cell. The cocoon, which is oval in shape, consists of particles of earth glued together and lined with a cement which renders it impervious to water and strong enough to resist considerable pressure without crushing. The insect passes the winter in the larval stage. However, the pupa begins to form in the fall. Specimens obtained during October showed the developing compound eyes and ocelli, while of examples secured the following April the adult characters of the head could be plainly seen through the skin, and their bodies were decidedly humped. One of these specimens which was kept in a cool room transformed to a pupa on or before April 23. Others obtained from an orchard on May 2, 1913, were all in the pupal stage, and one female pupa was partly colored.

NATURAL ENEMIES OF SAWFLY LEAF MINER[1]

A common enemy of the sawfly leaf miner is the chalcidid *Trichogramma minutum* Riley, which is an egg parasite. During the five years that

[1] Through the courtesy of Dr. L. O. Howard, the identifications of the parasites were made by Messrs. A. A. Girault and A. S. Rohwer, of the United States Bureau of Entomology.

Profenusa collaris has been under observation, *T. minutum* has twice made its appearance in conspicuous numbers in infested cherry orchards, in 1912 and in 1915. During the former year the larger percentage of the eggs of the leaf miner were attacked, and on some trees it was difficult to find an egg-bearing leaf which had not been visited by the parasite. In 1915 parasitism ranged from about 40 to 90 per cent on individual trees. Taking all trees into consideration, of the eggs deposited by the insect a larger percentage of them certainly failed to hatch than hatched, and for this mortality *T. minutum* appeared to be largely responsible.

The parasite was reared from both cherry and hawthorn foliage. The majority of the eggs of the leaf miner that were dissected contained a single parasite, and in only a few instances were twin larvæ or pupæ observed. On June 2, 1915, the parasites were all in the larval state, but on June 5, when the larvæ of *P. collaris* were beginning to abandon their mines in the foliage, about 50 per cent of the parasites were in the pupal state. By June 7 they had nearly all transformed to pupæ, and on June 9 the first adult appeared. During succeeding days the chalcidids appeared in large numbers, and the last specimen to make its appearance emerged on June 14. While the parasite was abundant about Geneva during this year, it was relatively quite scarce on plantings of *Crataegus* spp. at Rochester.

Besides the foregoing parasite there has been reared from *P. collaris* an ichneumon which proved to be a new species and has been listed by Rohwer [1] as "*Pezoporus tenthredinarum*." Apparently there is associated with this ichneumon an undescribed tryphonine, but owing to the small numbers collected it is impossible to make any definite statement at this time as to its status as a parasite of the sawfly leaf miner.

METHODS OF CONTROL

REMOVAL OF AFFECTED LEAVES

Of the operations systematically practiced, one that will probably prove most effective and economical in controlling the sawfly leaf miner is the picking of affected leaves. This species is peculiarly susceptible to this kind of repressive method, since there is only one brood of larvæ to attack the foliage, and oviposition extends over only a short period. The effect is that hatching of eggs and maturing of larvæ are, practically speaking, almost simultaneous for all of the creatures, and their activities during their injurious stages are therefore restricted to a relatively short period. By careful timing it is possible at a single picking to collect practically all of the larvæ by removing the affected leaves, which should then be burned to destroy the insects therein. The removal and destruction of all mined leaves, coupled with another practice—the destruc-

[1] Rohwer, S. A. Descriptions of new species of Hymenoptera. *In* Proc. U. S. Nat. Mus., v. 49, p. 216. 1915.

tion of wild hawthorns in the immediate vicinity of the cherry orchard—should leave few opportunities for the pest to develop to injurious numbers.

FUMIGATION WITH HYDROCYANIC-ACID GAS

Of the various measures employing insecticides tested by this station to protect cherry foliage from the work of the leaf miner, fumigation with hydrocyanic-acid gas alone was effective. Most cherry growers in New York are not equipped with suitable apparatus to undertake this means of affording protection to their trees, and fumigation should only be undertaken as an extreme measure and in an experimental way under expert direction.

CULTIVATION

Cultivation, if done with care and at the proper time, is destructive to many insects with subterranean habits. Species especially that undergo pupal development in the ground are not only peculiarly sensitive to disturbances of the soil, but plowing and cultivation, besides breaking up the cells of hibernating larvæ, exert another detrimental influence, exposing the helpless insects to insectivorous birds and other foes. Since it is the normal habit of the larvæ of this sawfly leaf miner to live in earthen cells for the greater portion of the life cycle of the species, such practices as fall or early spring plowing or cultivation are to be recommended from an entomological standpoint. These measures, fortunately, are standard operations which are invariably practiced by the most successful cherry growers.

DESTRUCTION OF UNCULTIVATED HOST PLANTS

The fact that the sawfly leaf miner is very partial to hawthorns, especially of the group *C. crus-galli*, and breeds most abundantly on them, suggests the desirability of destroying these plants when they exist in the immediate vicinity of a cherry orchard. The value of this operation is not known; but until there is more knowledge of the breeding habits of the pest the removal of wild plants along roadsides and hedgerows that are attractive to the insect for purposes of propagation would appear advisable as a precautionary measure.

SPRAYING OF HAWTHORNS

For the protection of hawthorns in decorative plantings, spraying seems to be preferred to any of the preceding measures. The insecticide which has given the most satisfactory results is composed of 1 pint of nicotine solution (40 per cent) to 100 gallons of water to which are added 4 pounds of soap. In making the treatment the liquid should be used in liberal amounts and applied with rather high pressures at the time when the insects first begin to mine the foliage.

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VARIATIONS IN MINERAL COMPOSITION OF SAP, LEAVES, AND STEMS OF THE WILD-GRAPE VINE AND SUGAR-MAPLE TREE

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INTRODUCTION

In a previous publication Kastle and the writer (9)[2] have shown the relation existing between the mineral components of the sap of the wild-grape vine (*Vitis cordifolia*) and those contained in the young leaves and stems at a certain period in its growth during the same year. At that time these writers stated that they did not know whether these relations would hold true throughout the growing season, and they purposed to continue the investigation so as to include the sap and other materials from different portions of this vine and other plants.

Since our former publication, the writer has found in the literature at hand that considerable work has been done by Chandler (1), Harris and Gortner (8), Dixon and others (2, 3, 4, 5, 6, 7) on the physiochemical properties of certain saps or plant juices, but, so far as we have been able to find, no work has been done on the mineral composition of the sap or on the changes occurring therein which might have any bearing on the above-mentioned investigation.

EXPERIMENTS WITH WILD-GRAPE VINE

With this idea in view, the writer has during the last three years (1912–1914) collected samples of the sap from the vine employed in the former work, in order to determine (1) whether the mineral composition of this sap varies at the same time in different parts of the vine, (2) whether it varies during a single season at a certain point, and (3) whether it varies during different years. The analyses are of interest, inasmuch as they show large differences in the composition of the sap, depending on the time and place of collection. The results are given in Tables I to XI and are expressed in percentage by weight, except where otherwise stated. The mineral components of the original sample have been calculated from the amounts found in the ash, except the chlorin, which was determined in the fresh sap. The sulphur-trioxid content of the original substance is probably low, since more or less sulphur is lost in ashing organic materials.

[1] The author desires to express his gratitude to Dr. J. H. Kastle, Director of the Kentucky Experiment Station, for his helpful advice during the progress of this investigation.

[2] Reference is made by number to "Literature cited," p. 541–542.

In order to understand more fully the different tabulations, a brief description of each sample follows.

Nos. 285, 812, and 852 were collected in April of 1912, 1913, and 1914, respectively, from the cut end of the same main branch about 20 feet from the root of the vine and just after the sap flow commenced.

No. 853 was collected in April, 1914, from the cut end of another main branch about 4 feet from the root of the vine and just after the sap flow commenced. This sample was taken at the same time as No. 852.

No. 854 was collected in April, 1914, from the same point as No. 852, but seven days later and just before the sap flow ceased.

No. 900 was collected in April, 1915, from the cut end of one of the main branches about 20 feet from the root of the vine and just after the sap flow commenced. This was a different branch from that from which No. 285 was taken, because no sap exuded from the old branch, and it seemed to have been greatly weakened by the annual loss.

No. 901 was collected in April, 1915, from several of the small branches or shoots which were of the previous year's growth and just after the sap flow commenced. This sample was taken at the same time as No. 900 and from 10 shoots which were located several feet from the main branches.

Nos. 902, 904, and 906 were collected for three successive days from 9 a. m. to 5 p. m., beginning on April 29, 1915, four days after and from the same point as No. 900.

Nos. 903, 905, and 907 were collected for three successive nights from 5 p. m. to 9 a. m., beginning on April 29, 1915, and from the same point as No. 900.

The variation in the percentage composition of the fresh sap and the ash of samples 852, 853, 900, and 901 are given in Tables I and II.

TABLE I.—*Variation in percentage composition of fresh sap collected at the same time from different points on the wild-grape vine* [1]

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Water at 100° C.	99.8279	99.8538	99.8183	99.8431	1 : 1.00	1 : 1.00
Organic matter	.1435	.1112	.1305	.1068	1 : .77	1 : .82
Silica (SiO_2)	.0001	.0001	.0003	.0017	1 : 1.00	1 : 5.67
Ferric and aluminic oxids (Fe_2O_3+ Al_2O_3)	.0001	.0001	.0001	.0004	1 : 1.00	1 : 4.00
Calcium oxid (CaO)	.0160	.0155	.0234	.0268	1 : .97	1 : 1.15
Magnesium oxid (MgO)	.0024	.0025	.0041	.0062	1 : 1.04	1 : 1.51
Sodium oxid (Na_2O)	.0012	.0012	.0010	.0011	1 : 1.00	1 : 1.10
Potassium oxid (K_2O)	.0050	.0112	.0167	.0074	1 : 2.24	1 : .44
Phosphorus pentoxid (P_2O_5)	.0015	.0026	.0030	.0030	1 : 1.73	1 : 1.00
Sulphur trioxid (SO_3)	.0019	.0017	.0025	.0033	1 : .89	1 : 1.32
Chlorin	.0004	.0001	.0001	.0002	1 : .25	1 : 2.00
Total	100.0000	100.0000	100.0000	100.0000	.....	.....
$d\frac{25°}{25°}$	1.0009	1.0008	1.00082	1.00027	1 : 1.00	1 : 1.00
Nitrogen as nitrates	.0013	.0024	.00004	.00001	1 : 1.85	1 : .25
Crude ash	.0384	.0477	.0700	.0662	1 : 1.24	1 : .95

[1] Nos. 852 and 853 were collected in 1914; Nos. 900 and 901 in 1915.

TABLE II.—*Percentage composition of ash of the samples in Table I*

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Silica (SiO_2)	0.339	0.231	0.485	2.505	1 : 0.68	1 : 5.16
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.261	.210	.143	.574	1 : .80	1 : 4.01
Calcium oxid (CaO)	41.628	32.535	33.432	40.386	1 : .78	1 : 1.21
Magnesium oxid (MgO)	6.364	5.296	5.828	9.424	1 : .83	1 : 1.62
Sodium oxid (Na_2O)	3.234	2.522	1.483	1.633	1 : .78	1 : 1.10
Potassium oxid (K_2O)	13.146	23.465	23.787	11.103	1 : 1.78	1 : .47
Phosphorus pentoxid (P_2O_5)	3.860	5.380	4.349	4.543	1 : 1.39	1 : 1.04
Sulphur trioxid (SO_3)	5.008	3.531	3.562	5.052	1 : .71	1 : 1.42
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	73.840	73.170	73.069	75.220	.....	.....

From an examination of Table I it is apparent that the water, calcium, and sodium content of the sap are fairly constant when collected at two different points at the same time during the same year, while the silica, iron, aluminum, potassium, phosphorus, and chlorin are the large variable constituents, depending on the time and point of collection. The organic matter is higher in the sap taken at a point on the main branch about 20 feet from the root than it is on the same branch closer to the ground or on the new branches. The silica, iron, aluminum, calcium, magnesium, and sulphur, however, are higher in the sap in the new branches. These facts agree with the writer's previous findings, which show that the minerals accumulate in the leaves. As the grapevine puts forth leaves every year only on the parts of more recent growth, the above results are what one would naturally expect when considered in connection with the former work.

Another interesting point is that certain constituents—namely, silica, iron, aluminum, magnesium, and phosphorus—may be about the same in the sap when collected from two different points at the same time during a given year, but vary widely when compared the following season.

A further point of interest is that while the ratio of calcium oxid to magnesium oxid is fairly constant in each sap of Table I, that of the potassium oxid to sodium oxid is variable, as shown in Table III.

TABLE III.—*Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of the wild grape collected at the same time from different points on the vine*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852	6.7 : 1	4.2 : 1
853	6.2 : 1	9.3 : 1
900	5.7 : 1	16.7 : 1
901	4.3 : 1	6.7 : 1

TABLE IV.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at different times during the same season* [1]

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902.[b]	Ratio between— Nos. 852 and 854.	Ratio between— Nos. 900 and 902.
Water at 100° C.	99.8279	99.7545	99.8183	99.7026	1 : 1.00	1 : 1.00
Organic matter	.1435	.1821	.1305	.2208	1 : 1.27	1 : 1.69
Silica (SiO_2)	.0001	.0003	.0003	.0007	1 : 3.00	1 : 2.33
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0001	.0001	.0001	.0003	1 : 1.00	1 : 3.00
Calcium oxid (CaO)	.0160	.0221	.0234	.0277	1 : 1.38	1 : 1.18
Magnesium oxid (MgO)	.0024	.0036	.0041	.0047	1 : 1.50	1 : 1.15
Sodium oxid (Na_2O)	.0012	.0013	.0010	.0011	1 : 1.08	1 : 1.10
Potassium oxid (K_2O)	.0050	.0277	.0167	.0316	1 : 5.54	1 : 1.89
Phosphorus pentoxid (P_2O_5)	.0015	.0045	.0030	.0069	1 : 3.00	1 : 2.30
Sulphur trioxid (SO_3)	.0019	.0037	.0025	.0036	1 : 1.95	1 : 1.44
Chlorin	.0004	.0001	.0001	.....	1 : .25	.....
Total	100.0000	100.0000	100.0000	100.0000	.....	.....
$d\frac{25°}{25°}$	1.0009	1.0007	1.00082	.....	1 : 1.00	.....
Nitrogen as nitrates	.0013	.0028	.00004	.....	1 : 2.15	.....
Crude ash	.0384	.0863	.0700	.1012	1 : 2.25	1 : 1.45

[1] Nos. 852 and 854 were collected in 1914; Nos. 900 and 902, in 1915.
[b] Composition by volume, but this does not appreciably affect the percentage by weight.

TABLE V.—*Percentage composition of ash of samples in Table IV*

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902.	Ratio between— Nos. 852 and 854.	Ratio between— Nos. 900 and 902.
Silica (SiO_2)	0.339	0.371	0.485	0.678	1 : 1.09	1 : 1.40
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.261	.093	.143	.254	1 : .36	1 : 1.78
Calcium oxid (CaO)	41.628	25.627	33.432	27.386	1 : .62	1 : .82
Magnesium oxid (MgO)	6.364	4.225	5.828	4.602	1 : .66	1 : .79
Sodium oxid (Na_2O)	3.234	1.486	1.483	1.078	1 : .46	1 : .73
Potassium oxid (K_2O)	13.146	32.080	23.787	31.198	1 : 2.44	1 : 1.31
Phosphorus pentoxid (P_2O_5)	3.860	5.269	4.349	4.771	1 : 1.37	1 : 1.56
Sulphur trioxid (SO_3)	5.008	4.271	3.562	3.564	1 : .85	1 : 1.00
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	73.840	73.422	73.069	73.531	.....	.....

In Table IV it appears that in both years there is a concentration of practically all the minerals in the sap at the end of the sap flow, or when new leaves develop, compared with the beginning. The ratio of increase of some of the minerals—namely, silica, iron, aluminum, potassium, phosphorus, and sulphur—in one or both years is much greater than the remainder. There is also a wide variation in the percentages of ash in the different samples, which partly accounts for some of these differences (Table V). Furthermore, an examination of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid shows that the former remains fairly constant, while the latter is variable and demon-

strates the large amount of potassium oxid in the sap at the end of the sap flow compared with the beginning, since the sodium oxid is fairly constant during both years. See Table VI.

TABLE VI.—*Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape taken from the same point on the vine at different times during the same season*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852	6. 7 : 1	4. 2 : 1
854	6. 1 : 1	21. 3 : 1
900	5. 7 : 1	16. 7 : 1
902	5. 9 : 1	28. 7 : 1

An examination of the minimum and maximum percentages of the minerals in the sap collected at the same point during four successive years and just after the sap flow commenced shows the largest variations which have been found (Table VII). The constituents vary in order of magnitude as follows: Potassium, chlorin, iron, aluminum, silica, phosphorus, sulphur, magnesium, sodium, and calcium. Again there is a wide variation in the ash content of the different samples (Table VIII).

TABLE VII.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at the beginning of the sap flow during four successive years*

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum.
Water at 100° C	99. 6340	99. 8665	99. 8279	99. 8183	1 : 1. 00
Organic matter	. 2782	. 0917	. 1435	. 1305	1 : 3. 03
Silica (SiO_2)	. 0005	. 0005	. 0001	. 0003	1 : 5. 00
Ferric and aluminic oxids ($Fe_2O_3 + Al_2O_3$)	. 0006	. 0002	. 0001	. 0001	1 : 6. 00
Calcium oxid (CaO)	. 0220	. 0206	. 0160	. 0234	1 : 1. 46
Magnesium oxid (MgO)	. 0044	. 0043	. 0024	. 0041	1 : 1. 83
Sodium oxid (Na_2O)	. 0017	. 0016	. 0012	. 0010	1 : 1. 70
Potassium oxid (K_2O)	. 0468	. 0112	. 0050	. 0167	1 : 9. 36
Phosphorus pentoxid (P_2O_5)	. 0058	. 0017	. 0015	. 0030	1 : 3. 87
Sulphur trioxid (SO_3)	. 0052	. 0016	. 0019	. 0025	1 : 3. 25
Chlorin	. 0008	. 0001	. 0004	. 0001	1 : 8. 00
Total	100. 0000	100. 0000	100. 0000	100. 0000	.....
$d\frac{25°}{25°}$	1. 0035	1. 00067	1. 0009	1. 00082	1 : 1. 00
Nitrogen as nitrates	. 0075	. 00048	. 0013	. 00004	1 : 187. 50
Crude ash	. 1130	. 0570	. 0384	. 07000	1 : 2. 94

TABLE VIII.—*Percentage composition of ash of samples in Table VII*

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum.
Silica (SiO_2)	0.405	0.809	0.339	0.485	1 : 2.39
Ferric and aluminic oxids (Fe_2O_3+ Al_2O_3)	.540	.387	.261	.143	1 : 3.78
Calcium oxid (CaO)	19.490	36.070	41.628	33.432	1 : 2.14
Magnesium oxid (MgO)	3.900	7.594	6.364	5.828	1 : 1.95
Sodium oxid (Na_2O)	1.500	2.742	3.234	1.483	1 : 2.16
Potassium oxid (K_2O)	41.380	19.617	13.146	23.787	1 : 3.15
Phosphorus pentoxid (P_2O_5)	5.090	3.059	3.860	4.349	1 : 1.66
Sulphur trioxid (SO_3)	4.590	2.742	5.008	3.562	1 : 1.83
Carbon dioxid, not determined	.....	.....	.....	.....	.....
Total	76.895	73.020	73.840	73.069	.....

As stated before, Nos. 285, 812, and 852 were collected from the same branch, whereas No. 900 was taken an equal distance from the root on another branch, as the former was so greatly weakened that no sap exuded from it at the proper time, although new growth came on it later, showing that it was not dead. If a comparison now be made of Nos. 285, 812, and 852, it will be found that there has been a marked reduction in practically all of the mineral substances in the sap in the two succeeding years compared with the first, and, moreover, this was very sharp in some constituents in the second and, in others, in the third year. Furthermore, it will be noticed that among those which show a decided decrease in the second year are potassium and phosphorus, both of which are included among the chief essential plant-food elements.

According to the different analyses of the sap, potassium is among the high mineral constituents, and as this element has shown the largest loss, this may account for the weakened condition of the branch.

The ratios of calcium oxid to magnesium oxid and of potassium oxid to sodium oxid in the various samples of Table VII are as given in Table IX.

TABLE IX.—*Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape from the same point on the vine at the beginning of the sap flow during four successive years*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
285	5.0 : 1	27.5 : 1
812	4.8 : 1	7.0 : 1
852	6.7 : 1	4.2 : 1
900	5.7 : 1	16.7 : 1

Table IX shows that the ratio of calcium oxid to magnesium oxid is fairly constant in the different samples, while the wide variation in the potassium oxid and sodium oxid from 27.5 in 1912 to 4.2 in 1914 would indicate that these figures were obtained from the sap of different plants rather than from that of the same vine at different times.

TABLE X.—*Variation in percentage composition*[1] *of fresh sap of wild grape collected for three successive days and nights*[2]

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Water at 100° C.	99.7026	99.7354	99.7436	99.7473	99.7592	99.7469	1 : 1.00
Organic matter	.2208	.1971	.1766	.1892	.1732	.1874	1 : 1.27
Silica (SiO_2)	.0007	.0006	.0008	.0007	.0005	.0007	1 : 1.60
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0003	.0002	.0005	.0001	.0005	.0001	1 : 5.00
Calcium oxid (CaO)	.0277	.0248	.0248	.0245	.0228	.0233	1 : 1.21
Magnesium oxid (MgO)	.0047	.0042	.0089	.0049	.0070	.0045	1 : 2.12
Sodium oxid (Na_2O)	.0011	.0008	.0039	.0008	.0041	.0014	1 : 5.13
Potassium oxid (K_2O)	.0316	.0279	.0296	.0239	.0245	.0254	1 : 1.32
Phosphorus pentoxid (P_2O_5)	.0069	.0060	.0077	.0056	.0054	.0067	1 : 1.43
Sulphur trioxid (SO_3)	.0036	.0030	.0036	.0030	.0028	.0036	1 : 1.29
Chlorin, not determined	.....	.....	.....	.....	.....	.....	.....
Total	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	.....
Crude ash	.1012	.0916	.0925	.0843	.0780	.0839	1 : 1.30

[1] By volume.
[2] Nos. 902, 904, and 906 were collected on successive days; Nos. 903, 905, and 907 were collected on successive nights.

TABLE XI.—*Percentage composition of ash of samples in Table X*

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Silica (SiO_2)	0.678	0.691	0.884	0.804	0.641	0.788	1 : 1.38
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.254	.267	.590	.079	.641	.110	1 : 8.11
Calcium oxid (CaO)	27.386	27.068	26.820	29.034	29.167	27.796	1 : 1.09
Magnesium oxid (MgO)	4.602	4.621	9.607	5.840	8.939	5.366	1 : 2.09
Sodium oxid (Na_2O)	1.078	.823	4.220	1.006	5.269	1.672	1 : 6.40
Potassium oxid (K_2O)	31.198	30.478	31.986	28.277	31.430	30.232	1 : 1.13
Phosphorus pentoxid (P_2O_5)	6.771	6.544	8.271	6.604	6.950	7.999	1 : 1.26
Sulphur trioxid (SO_3)	3.564	3.261	3.841	3.524	3.628	4.327	1 : 1.33
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....	.....
Total	75.531	73.753	86.219	75.168	86.665	78.290	.....

Referring to the results in Table X, it will be seen that there is a considerable variation occurring daily in the mineral composition of the sap and that, as a rule, most of its constituents are present in larger amounts during the day, while, on the other hand, its composition is more constant at night (Table XI).

As there was such a wide variation in short periods of time in the composition of the sap of this vine, it was thought desirable to collect further samples of the young leaves and stems in order to determine if this would hold true in regard to these parts. Accordingly, in June, 1915, or two months after the sap was first collected, and every two weeks thereafter for six weeks, samples of the succulent young stems and leaves representing the same stage of growth were taken. Therefore, the results are somewhat comparable with each other and with those formerly obtained, since the earlier samples were taken in a similar manner in Nos. 908 and 909. The consecutive analyses are given in Tables XII to XV along with those of Nos. 627 and 628 of 1912.

TABLE XII.—*Variation in percentage composition of young green leaves of wild-grape vine in the same and in different years*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Water at 100° C.	75.4700	75.1200	71.1975	73.1525	72.6015	1:1.06
Organic matter	22.8500	23.3181	26.8851	25.3750	25.9097	1:1.18
Silica (SiO_2)	.1372	.0514	.0642	.0291	.0537	1:4.71
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0214	.0197	.0394	.0117	.0165	1:3.37
Calcium oxid (CaO)	.7200	.5478	.7585	.5515	.4427	1:1.71
Magnesium oxid (MgO)	.1337	.1112	.1333	.1004	.1078	1:1.33
Sodium oxid (Na_2O)	.0356	.0167	.0214	.0236	.0287	1:2.13
Potassium oxid (K_2O)	.3427	.5619	.5943	.5076	.5752	1:1.73
Phosphorus pentoxid (P_2O_5)	.2260	.2104	.2020	.1891	.1992	1:1.20
Sulphur trioxid (SO_3)	.0634	.0428	.1043	.0595	.0650	1:2.44
Total	100.0000	100.0000	100.0000	100.0000	100.0000	.....
Crude ash	2.3300	2.0140	2.4595	1.9530	1.8790	1:1.31

TABLE XIII.—*Percentage composition of ash of samples in Table XII*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Silica (SiO_2)	5.890	2.550	2.610	1.490	2.860	1 : 3.95
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.920	.980	1.600	.600	.880	1 : 2.67
Calcium oxid (CaO)	30.900	27.200	30.840	28.240	23.560	1 : 1.31
Magnesium oxid (MgO)	5.740	5.520	5.419	5.143	5.737	1 : 1.12
Sodium oxid (Na_2O)	1.530	.827	.870	1.209	1.527	1 : 1.85
Potassium oxid (K_2O)	14.710	27.899	24.163	25.992	30.613	1 : 2.08
Phosphorus pentoxid (P_2O_5)	9.700	10.447	8.215	9.682	10.600	1 : 1.29
Sulphur trioxid (SO_3)	2.720	2.127	4.239	3.046	3.457	1 : 1.99
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	72.110	77.550	77.956	75.402	79.234	.....

TABLE XIV.—*Variation in percentage composition of young green stems of wild-grape vine in the same and in different years*

Constituent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Water at 100° C.	79.2500	84.2750	81.9385	83.3210	82.6645	1 : 1.06
Organic matter.	20.0437	14.8654	17.0893	15.7397	16.4453	1 : 1.35
Silica (SiO_2)	.0041	.0048	.0069	.0037	.0040	1 : 1.86
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0003	.0051	.0038	.0041	.0032	1 : 17.00
Calcium oxid (CaO)	.1114	.1558	.2244	.2488	.1792	1 : 2.23
Magnesium oxid (MgO)	.0346	.0539	.0642	.0567	.0557	1 : 1.86
Sodium oxid (Na_2O)	.0171	.0078	.0133	.0158	.0093	1 : 2.19
Potassium oxid (K_2O)	.3883	.4813	.5154	.4846	.5098	1 : 1.33
Phosphorus pentoxid (P_2O_5)	.1277	.1055	.1078	.1010	.1009	1 : 1.27
Sulphur trioxid (SO_3)	.0228	.0454	.0364	.0246	.0281	1 : 1.99
Total	100.0000	100.0000	100.0000	100.0000	100.0000	.....
Crude ash	1.0200	1.1490	1.2810	1.3790	1.2175	1 : 1.35

TABLE XV.—*Percentage composition of ash of samples in Table XIV*

Constitutent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Silica (SiO_2)	0.400	0.420	0.540	0.270	0.330	1 : 2.00
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.030	.440	.300	.300	.260	1 : 14.67
Calcium oxid (CaO)	10.920	13.560	17.520	18.040	14.720	1 : 1.74
Magnesium oxid (MgO)	3.390	4.694	5.013	4.115	4.578	1 : 1.48
Sodium oxid (Na_2O)	1.680	.679	1.039	1.145	.764	1 : 2.47
Potassium oxid (K_2O)	38.070	41.892	40.241	35.140	41.876	1 : 1.19
Phosphorus pentoxid (P_2O_5)	12.520	9.184	8.419	7.322	8.291	1 : 1.71
Sulphur trioxid (SO_3)	2.240	3.951	2.840	1.784	2.305	1 : 2.21
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	69.250	74.820	75.912	68.116	73.124	.....

In Tables XII and XIV it will be found that the ratio between the lowest and the highest result obtained for each of the other mineral constituents in the several samples of leaves and stems is more constant than it is for the silica, iron, and aluminum. It will further be found that the results obtained this year on the different samples corroborate, except in one instance, those of 1912 in showing that there is a concentration of all the constituents in the leaf compared with the stem, and the exception is that whereas the potassium content of the stem in 1912 was greater than in the leaf, this year (1915) it was less in every case.

In the leaf the silica, sodium, magnesium, and phosphorus are uniformly lower than in 1912 and the organic matter and potassium are higher, while the other constituents vary both above and below the former results. In the stem, however, the organic matter, sodium, and phosphorus are lower than formerly and the iron, aluminum, calcium, magnesium, potassium, and sulphur are higher, while the silica is variable.

Another interesting point is that most of the results show that the mineral constituents are lower in the leaves of this year (1915) than formerly, while in the stem they are higher.

The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the leaf and stem below show, as did those of the sap, that the former is more constant than the latter (Table XVI).

TABLE XVI.—*Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in leaves and stems of young wild-grape vine in the same and in different years*

Part and sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
Leaf:		
627	5.4 : 1	9.6 : 1
908	4.9 : 1	33.6 : 1
910	5.7 : 1	27.8 : 1
912	5.5 : 1	21.5 : 1
914	4.1 : 1	20.0 : 1
Stem:		
628	3.2 : 1	22.7 : 1
909	2.9 : 1	61.7 : 1
911	3.5 : 1	38.8 : 1
913	4.4 : 1	30.7 : 1
915	3.2 : 1	54.8 : 1

EXPERIMENTS WITH SUGAR MAPLE

Having found such a wide variation in the composition of the sap of the wild-grape vine, it was thought that it might prove of further interest to compare the analyses of the sap of the same sugar-maple tree (*Acer saccharum*) collected during two successive years. Accordingly, early in 1913 and 1914, just after the sap began to rise, samples were collected at the same point on the tree, about 3 feet from the ground.

Also, for a further comparison, the sap was collected in 1913, just after the sap flow commenced, from a water-maple tree (*Acer saccharinum*) at a point about 10 feet from the ground.

The results are given in Tables XVII and XVIII.

TABLE XVII.—*Variation in composition of the sap of the water-maple and sugar-maple trees*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio between Nos. 776 and 851.
		No. 776.[a]	No. 851.[b]	
Water at 100° C.	98.2035	98.2953	98.3227	1 : 1.00
Organic matter	1.7677	1.6812	1.6247	1 : .97
Silica (SiO_2)	.0013	.0016	.0011	1 : .69
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0001	.0001	.0001	1 : 1.00
Calcium oxid (CaO)	.0053	.0097	.0200	1 : 2.06
Magnesium oxid (MgO)	.0009	.0018	.0026	1 : 1.44
Sodium oxid (Na_2O)	.0020	.0004	.0009	1 : 2.25
Potassium oxid (K_2O)	.0118	.0084	.0178	1 : 2.12
Phosphorus pentoxid (P_2O_5)	.0023	.0007	.0060	1 : 8.57
Sulphur trioxid (SO_3)	.0004	.0002	.0033	1 : 16.50
Chlorin	.0047	.0006	.0008	1 : 1.33
Total	100.0000	100.0000	100.0000	.....
$d\frac{25°}{25°}$	1.0056	1.0045	1.0059	1 : 1.00
Nitrogen as nitrates	.0007	.....	.....	.....
Crude ash	.0296	.0336	.0678	1 : 2.02

[a] Collected in 1913 just after the sap flow commenced.
[b] Collected in 1914 just after the sap flow commenced, from same point on the same tree as No. 776.

TABLE XVIII.—*Percentage composition of ash of samples in Table XVII*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio between Nos. 776 and 851.
		No. 776.	No. 851.	
Silica (SiO_2)	4.444	4.868	1.701	1: .35
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.506	.237	.220	1: .93
Calcium oxid (CaO)	18.003	28.864	29.561	1:1.02
Magnesium oxid (MgO)	2.926	5.399	3.893	1: .72
Sodium oxid (Na_2O)	6.751	1.241	1.352	1:1.09
Potassium oxid (K_2O)	39.945	24.902	26.211	1:1.05
Phosphorus pentoxid (P_2O_5)	7.651	2.079	8.881	1:4.27
Sulphur trioxid (SO_3)	1.238	.518	4.834	1:9.33
Carbon dioxid, not determined	.....	.....	.....	.....
Total	81.464	68.108	76.653	.....

In Table XVII we find that the calcium, magnesium, sodium, potassium, phosphorus, and sulphur are much higher in the sugar-maple sap in 1914 than in 1913 and the silica is lower, while the water, organic matter, iron, and aluminum are about the same in both years. The largest varying constituents are sulphur and phosphorus.

Again, on comparing the sap of the sugar maple with that of the water maple, there are found large differences in the calcium, magnesium, sodium, potassium, phosphorus, sulphur, and chlorin, while the water, organic matter, silica, iron, and aluminum are about the same.

The large amount of sodium and chlorin in the sap of the water maple may be explained as due to the fact that this tree was located on a city lot and may have received sodium chlorid from the drainage, while the sugar maple was located in the country. On the other hand, the wild-grape vine was also on a city lot, but in a different locality, and its sap did not show a large chlorin content; still, this may have been due to the difference in the drainage of the two places.

The differences obtained in the mineral constituents of the several samples of sap can not be due altogether to the different moisture content of the soil, for the large variations in the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the tables show that it is not a dilution of the sap by the soil water.

The moisture content of the soil at the time of the sap collection was not determined, and, of course, this would be influenced by several factors, such as temperature, rainfall, sunshine, and wind at that period. Taking into account the rainfall alone will not explain the differences obtained, as will be seen from Table XIX.

TABLE XIX.—*Rainfall in inches during four successive years*

Month.	1912.	1913.	1914.	1915.
January	1.78	10.35	2.50	4.38
February	2.50	2.61	3.87	1.12
March	4.36	6.04	2.24	1.49
April	6.89	2.41	2.23	.65

During the spring of this year (1915) there was less rainfall in this vicinity than for years, and there is no doubt that the moisture content of the soil at the time of the sap collection in 1915 was considerably lower than it was the three preceding years. If the results are to be explained from the dilution standpoint, then those of Nos. 285 and 900 in Table VII should be in harmony with what has just been stated, while, as a matter of fact, they are contradictory, except for one constituent.

The foregoing results show that the sap has a variable mineral composition which later on influences the structure of the growing parts, and this undoubtedly explains the differences in composition of the same and different varieties of plants.

SUMMARY

(1) There is considerable variation in the composition of the sap of the wild-grape vine when collected at the same time from two different points. This has been the case for two seasons.

(2) Large differences in the composition of this sap were found when it was collected at the same point on the vine at different times during the same season. The minerals in the sap are higher at the end of the

sap flow than at the beginning. This has also been proved for two seasons.

(3) The widest variations in the composition of this sap were found when it was collected at the same point on a main branch of the vine at the beginning of the sap flow during four successive years. The periodic loss of sap greatly weakened this branch, and there was also a steady decline in the mineral components of the sap taken from it, particularly potassium and phosphorus.

(4) There was found a considerable variation occurring daily in the composition of this sap. The mineral constituents were generally higher during the day and the sap had a more uniform composition during the night.

(5) The young leaves and stems of this vine at the same stage of growth were also found to vary considerably in composition during different years and also in the same season.

(6) The sap of the same sugar-maple tree was found to vary widely in composition when collected at the same point on the tree during two successive years just after the sap flow had commenced.

(7) The mineral composition of the sap of the water-maple tree was found to be different from that of the sugar maple.

(8) The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid, together with other factors, demonstrate that the differences in composition can not be altogether explained as being due to a dilution of the sap from the water in the soil.

(9) It has been shown that the sap has a variable mineral composition which influences the structure of the growing parts and undoubtedly explains the differences in composition of the same and different varieties of plants.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., DECEMBER 27, 1915 NO. 13

CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES

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INTRODUCTION

In a former paper[1] embodying a study of the general course of the carbohydrate transformations in sweet potatoes (*Ipomoea batatas*) during storage, certain data were presented indicating that the sugar content of sweet potatoes remains comparatively low while they are in the ground, but that immediately after the roots are harvested there is a transformation of starch into sugar which takes place more rapidly at that time than at subsequent periods. It was pointed out that this initial transformation seemed to be not greatly affected by temperature, but seemed rather to depend upon internal factors. It was suggested that possibly this initial change was associated with the cessation of the activity of the leaves. In view of the fact that this initial change appeared to be a phase of the carbohydrate metabolism of the sweet potato which was inaugurated only under certain conditions and which differed in some respects from subsequent changes, it seemed worth while to investigate this process more fully in order to make out something, if possible, as to its nature by a study of its progress at different temperatures.

EXPERIMENTATION IN CARBOHYDRATE TRANSFORMATION

PLAN OF EXPERIMENTS

The plan carried out in this work was, in general, to compare the carbohydrate transformations taking place in sweet potatoes during a period of 10 or 12 days immediately after they had been dug with the changes taking place during a second subsequent period of equal length. These experiments were carried out at temperatures of 30°, 15.5°, and 5° C.

[1] Hasselbring, Heinrich, and Hawkins, L. A. Physiological changes in sweet potatoes during storage. *In* Jour. Agr. Research, v. 3, no. 4, p. 331–342. 1915. Literature cited, p. 341–342.

The sweet potatoes used in the first series of experiments were dug on September 30, thoroughly washed, and were kept covered in the laboratory until the following day. In the further manipulation each potato was split lengthwise into two parts as nearly equal as possible. So far as could be determined the potatoes were cut longitudinally in a dorsiventral plane. One half, marked "a," was ground immediately, and samples were taken from the mash for the determination of moisture, sugar, and starch. The other half, marked "b," was stored. Six halves were stored at each temperature, the corresponding halves having been grated and sampled as described. The operation of preparing and sampling the halves for a set of experiments at the three temperatures required two days. Simultaneously with the halves a number of whole sweet potatoes were put into the constant-temperature chambers in which the experiments were conducted. At the end of 12 days the stored halves were taken out, grated, and sampled. After the completion of this operation, which required two days, the stored whole potatoes (which during this time had been subjected to the same conditions as the stored halves) were split lengthwise, like the first set, and one half was prepared for analysis. The other half was stored for another period of 12 days, after which it also was grated and analyzed. It will thus be noted that the difference in composition of the two halves of the first set of roots showed the change during the first period of 12 days immediately after the potatoes had been dug, while the difference in composition of the two halves of the second lot showed the change for a second period of 12 days immediately following the first period.

Although the time during which the sweet potatoes were exposed to the experimental conditions was essentially the same for the comparable lots, some unimportant differences necessarily crept in. Thus, for instance, it was impossible to prepare a complete set in a single day; therefore, one half of the potatoes used in the experiment were prepared one day and the other half the following day. Consequently, the one lot remained in the laboratory about a day longer than the other. Also, although the different groups were taken out of their respective chambers in the same order in which they were put in, no attention was paid to the order in which the individual potatoes were removed, since it was necessary to work as rapidly as possible. On this account it is likely that some halves remained in the chambers a few hours longer and others a few hours less than the assigned period, but it is obvious, considering the slowness of the changes that take place, that these discrepancies can have no effect on the general result.

The whole sweet potatoes stored simultaneously with the first set of halves also remained in the chambers two days longer than the halves, on account of the time required to grind and sample the stored halves; but this also is of no consequence, since the object of the experiments was

to compare the changes in the roots during the period immediately after they were dug with those during a subsequent period of the same length.

The second series of experiments was in all respects like the first except that the potatoes were dug on October 16 and placed in the experimental chambers on October 17 and 18. The length of time of storage was 12 days.

It was the object of the third series of experiments to determine the effect of removal of the vines on the initial carbohydrate changes in the sweet potato. The potatoes used in this series were, therefore, not dug until some time after the vines had been killed. The first frost, which killed the leaves but not the vines, occurred on October 22; a few days later, October 27, the vines were cut off close to the ground, so that from this time there would be no further transfer of materials from the vines to the roots. The potatoes were dug on November 6 and were thereafter treated as described for the other experiments, with the exception that the storage period was 10 days.

METHODS OF ANALYSIS

The methods of analysis were essentially the same as formerly described.[1] Only a few exceptions need be noted. The samples for moisture determinations were covered with 95 per cent alcohol as before, but the alcohol was evaporated in a drying oven at 50° C. Thereupon the samples were dried to their lowest weight in a vacuum oven in a slow current of air. This procedure gave clean, nearly white samples. For the starch determinations 10 gm. were weighed out and the whole sample, instead of an aliquot, was extracted, ground, and used for hydrolysis. The sugar samples were put into flasks, which were then nearly filled with 70 per cent alcohol, with the addition of a little calcium carbonate, and boiled for a minute or two. The starch samples were stored, without boiling, in 95 per cent alcohol.

GENERAL OBSERVATIONS ON THE EXPERIMENTS

In the experiments described halves of the same sweet potato were compared with each other, the one being analyzed immediately and the other at the end of a 10 to 12 day period of storage. Two questions immediately arise regarding this procedure, which was adopted because different sweet potatoes of the same variety differ much in composition: First, are the halves of the same potato alike in composition; and, second, do the cut potatoes behave in the same manner as whole potatoes in storage?

Müller-Thurgau[2] in his work on the common Irish potato found that there were only slight differences in the sugar content of the two halves

[1] Hasselbring, Heinrich, and Hawkins, L. A. Physiological changes in sweet potatoes during storage. *In* Jour. Agr. Research, v. 3, no. 4, p. 331–342. 1915. Literature cited, p. 341–342.

[2] Müller, Hermann, *Thurgau*. Ueber Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Ein Beitrag zur Kenntniss des Stoffwechsels der Pflanzen. *In* Landw. Jahrb., Bd. 11, p. 751–828, pl. 26. 1882.

of potatoes cut lengthwise; it was therefore somewhat astonishing to find considerable differences in the two halves of sweet potatoes which were examined in July and August and which had been stored up to that time from the previous year. On this account further examinations were made of freshly dug sweet potatoes and of others of the same crop which had been stored in the chambers with the experimental sweet potatoes. The results of these examinations are given here.

On September 9 three freshly dug sweet potatoes were split dorsiventrally as nearly as could be judged. While one half was being prepared for analysis the other was kept wrapped in a damp cloth. From the mash of each grated half two samples were weighed out for sugar determinations, two for starch, and two for moisture. From each of the sugar samples and from each of the starch samples two determinations were made. The results calculated in percentages are given in Table I. The halves of the same potatoes are indicated by "a" and "b." The data afford an opportunity to estimate the error that is likely to occur in duplicate determinations in one sample, the error in sampling, and also the difference in composition of the halves of the same potato. The data show that the longitudinal halves of freshly dug sweet potatoes have very nearly the same composition and that errors due to method and technique are negligible.

TABLE I.—*Percentage composition of halves of freshly dug sweet potatoes*

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Cane sugar.	Starch.
1a	71.75	0.66	2.27	21.00
		.66	2.23	20.99
1a	71.80	.68	2.20	21.00
		.68	2.25	20.95
1b	71.23	.79	2.17	21.42
		.78	2.21	21.47
1b	71.29	.75	2.21	21.43
		.77	2.25	21.53
2a	73.53	.87	2.01	19.58
		.85	2.00	19.47
2a	73.55	.90	1.95	19.69
		.90	1.93	19.55
2b	74.47	.94	1.95	18.32
		.95	2.03	18.44
2b	74.32	.98	2.00	18.28
		.96	2.01	18.16
3a	73.04	1.13	1.74	19.59
		1.15	1.68	19.78
3a	73.08	1.16	1.68	19.60
		1.16	1.60	19.58
3b	73.29	.....	.....	19.33
		.....	.....	19.39
3b	73.14	1.29	1.71	19.21
		1.32	1.66	19.25

To determine the effect of storage on the composition of different parts of the same sweet potato a number of other potatoes which had been stored for various lengths of time under different conditions were examined. One set of three potatoes was kept in the laboratory for four days. The other sets were stored for a month in the different chambers with the experimental potatoes.

In this case two samples were taken from each half, but the determinations were not made in duplicate for each sample. The results of these analyses are collected in Table II.

TABLE II.—*Percentage composition of halves of sweet potatoes kept for various times under different conditions*

KEPT IN LABORATORY FROM NOV. 6 TO NOV. 10

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Percentage of difference between halves.	Cane sugar.	Percentage of difference between halves.	Starch.	Percentage of difference between halves.
148a	74.64	1.21	9.9	3.01	2.6	16.36	1.4
	74.58	1.21		3.06		16.33	
148b	74.46	1.09		2.97		16.53	
	74.52	1.09		2.94		16.61	
149a	76.48	1.41	4.3	3.51	2.4	13.93	8.4
	76.48	1.40		3.52		14.09	
149b	75.49	1.36		3.43		15.22	
	75.48	1.33		3.43		15.16	
150a	76.32	.82	16.4	3.40	.3	14.45	3.4
	76.28	.83		3.36		14.68	
150b	76.72	.96		3.38		14.20	
	76.69	.96		3.40		13.93	

STORED IN 30° C. CHAMBER FROM NOV. 7 TO DEC. 5

110a	73.47	0.74	14.1	4.72	6.3	15.49	3.9
	73.44	.75		4.69		15.54	
110b	72.62	.64		4.97		16.21	
	72.52	.64		5.03		16.04	
111a	72.29	.70	6.3	4.82	2.2	16.79	3.1
	72.27	.72		4.78		16.57	
111b	72.06	.76		4.93		17.27	
	72.01	.75		4.88		17.14	

STORED IN 15.5° C. CHAMBER FROM OCT. 17 TO NOV. 11

68a	71.83	1.18	1.7	3.71	7.0	18.22	2.3
	71.84	1.16		3.73		18.21	
68b	70.99	1.16		3.98		18.71	
	70.98	1.14		3.98		18.54	
69a	71.96	1.54	4.2	3.79	1.3	17.80	6.7
	71.92	1.54		3.79		17.82	
69b	73.52	1.47		3.73		16.64	
	73.49	1.48		3.75		16.61	

TABLE II.—*Percentage composition of halves of sweet potatoes kept for various times under different conditions*—Continued

STORED IN 5° C. CHAMBER FROM OCT. 17 TO NOV. 11

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Percentage of difference between halves.	Cane sugar.	Percentage of difference between halves.	Starch.	Percentage of difference between halves.
89a	76.30	2.35	5.5	4.35	2.5	12.02	3.9
	76.32	2.35		4.38		12.02	
89b	75.79	2.22		4.48		12.38	
	75.71	2.22		4.47		12.60	
95a	75.98	2.61	2.5	3.97	7.2	12.44	.4
	75.97	2.61		3.95		12.40	
95b	76.16	2.57		3.68		12.48	
	76.21	2.52		3.67		12.45	

The results indicate that the longitudinal halves of sweet potatoes which have been stored for a time are likely to show a greater dissimilarity in composition than the halves of freshly dug potatoes. The differences, however, are not sufficiently great to overshadow the significant differences seen in the later tables. The inequality in composition of the halves of the same potato is much less than the unlikeness of different potatoes. The method of comparison of halves is therefore more satisfactory than the comparison of different whole potatoes unless a sufficient number be used to obliterate, to a great extent at least, errors due to individual differences.

The question whether the cut halves behave in the same way in storage as whole sweet potatoes can be more easily discussed in connection with the data presented later. It should be mentioned here, however, that in the first experiment at 15.5° C. the halves lost an unusual quantity of moisture and that this drying may have had some influence on their behavior. In subsequent experiments precautions were taken to avoid a loss of moisture as far as possible.

EXPERIMENTAL DATA

The data relating to all the experiments are collected in Tables III, IV, and V. Table III contains the data of the three experiments conducted at 30°, Table IV those of the experiments at 5°, and Table V those of the experiments at 15.5° C. Under each experiment the first section refers to the changes in composition of the sweet potatoes during the first period of 10 to 12 days immediately after the roots were dug, while the second section gives the changes during a period of equal length immediately following. The change during each period is shown by the difference in composition between the "a" halves analyzed at the beginning of their respective periods and the "b" halves of the same potatoes analyzed at the ends of the periods. The data in each case are based on the water content of the first half of the potato analyzed. The columns of differences show, respectively, the difference in the percentage of

reducing sugar, cane sugar, and starch in the half of the potato analyzed at the beginning, and the corresponding half analyzed at the end of the same period. These differences therefore represent the increments in the percentage of these substances in the stored halves during the 10- or 12-day storage period.

In the discussion of these tables it will be most convenient to compare the results of the experiments conducted at 30° with those of the experiments conducted at 5° and to consider later the experiments at 15.5° C.

The first and second experiments carried out at 30° (Table III) are similar in plan and execution and the results are entirely congruous, so that they may be discussed together. In both of these experiments there is a marked loss of starch during the first period of 12 days following the digging of the potatoes, but very little further loss during the second period. The changes in cane sugar correspond inversely to the changes in starch. During the first period there is a large increase in cane sugar, but during the second period there is almost no further gain. The figures showing the changes in reducing sugar during the first period are irregular, but during the second period there is a consistent and well-marked loss.

TABLE III.—*Changes in composition of sweet potatoes at 30° C.*

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
4a	74.02	0.47	0.37	2.63	1.95	18.91	−3.32
4b	68.63	.84		4.58		15.59	
5a	73.11	.79	.23	2.38	1.68	19.44	−2.42
5b	70.41	1.02		4.06		17.02	
6a	73.73	.91	−.26	2.09	2.43	19.16	−3.05
6b	66.38	.65		4.52		16.11	
7a	73.91	.80	−.06	2.22	2.63	18.88	−3.26
7b	65.71	.74		4.85		15.62	
8a	73.12	.81	.12	2.52	1.30	19.36	−1.93
8b	69.70	.93		3.82		17.43	
9a	74.06	.93	−.32	1.81	2.74	18.66	−3.06
9b	65.26	.61		4.55		15.60	

FIRST EXPERIMENT (SECOND PERIOD)

13a	71.10	1.41	−0.89	3.62	0.53	18.84	+0.13
13b	64.10	.52		4.15		18.97	
14a	72.52	1.23	−.74	3.61	.87	17.93	−.37
14b	66.50	.49		4.48		17.56	
15a	71.58	1.21	−.81	3.86	.79	18.23	−.09
15b	65.01	.40		4.65		18.14	
20a	74.15	1.38	−.72	3.79	.74	15.63	+.33
20b	66.14	.66		4.53		15.96	
21a	72.57	1.24	−.81	4.00	.88	17.49	−.52
21b	63.55	.43		4.88		16.97	
23a	73.25	1.44	−.86	3.36	.08	17.05	.75
23b	66.78	.58		3.44		17.80	

TABLE III.—*Changes in composition of sweet potatoes at 30° C.*—Continued

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
		SECOND EXPERIMENT (FIRST PERIOD)					
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
61a	76.39	0.35	0.12	1.71	1.76	16.09	−1.91
61b	72.21	.47		3.47		14.18	
62a	73.35	.89	−.01	1.93	1.90	19.35	−1.89
62b	71.63	.88		3.83		17.46	
63a	73.12	.71	.12	2.26	1.94	19.42	−2.22
63b	68.86	.83		4.20		17.20	
79a	72.48	.73	−.06	2.43	2.81	20.19	−3.20
79b	69.48	.67		5.24		16.99	
80a	73.88	.78	−.25	2.64	1.54	18.60	−1.41
80b	66.98	.53		4.18		17.19	
81a	74.20	.69	−.14	2.42	2.18	18.15	−2.49
81b	66.21	.55		4.60		15.66	
		SECOND EXPERIMENT (SECOND PERIOD)					
75a	70.74	0.94	−0.31	3.73	0.75	20.11	−0.76
75b	70.43	.63		4.48		19.35	
76a	73.67	1.26	−.37	3.74	.58	16.87	.01
76b	71.91	.89		4.32		16.88	
77a	74.19	.90	−.14	4.05	.72	16.26	−.74
77b	73.69	.76		4.77		15.52	
97a	73.58	1.17	−.33	3.63	1.36	17.25	−1.11
97b	72.80	.84		4.99		16.14	
98a	73.67	1.10	−.18	3.83	1.28	16.93	−.99
98b	72.65	.92		5.11		15.94	
99a	74.20	1.23	−.38	3.81	.51	16.16	−.06
99b	71.44	.85		4.32		16.10	
		THIRD EXPERIMENT (FIRST PERIOD)					
100a	74.78	0.86	0.02	2.76	1.25	17.15	−1.40
100b	72.65	.88		4.01		15.75	
101a	77.68	.56	.17	2.63	1.06	14.41	−1.69
101b	76.64	.73		3.69		12.72	
102a	74.43	.82	.11	3.39	1.29	17.41	−1.49
102b	73.25	.93		4.68		15.92	
103a	76.38	.75	.26	3.21	1.25	15.51	−1.81
103b	75.83	1.01		4.46		13.70	
104a	75.72	.77	.28	2.78	1.07	16.25	−1.38
104b	74.48	1.05		3.85		14.87	
105a	75.54	.96	.17	3.27	.77	16.10	−1.24
105b	74.99	1.13		4.04		14.86	
		THIRD EXPERIMENT (SECOND PERIOD)					
109a	70.69	0.76	−0.23	4.53	0.34	19.10	0.05
109b	70.31	.53		4.87		19.15	
112a	74.76	1.61	−.55	4.32	.47	14.82	.02
112b	74.26	1.06		4.79		14.84	
113a	74.17	.94	−.24	4.42	.26	15.87	−.18
113b	72.95	.70		4.68		15.69	
114a	75.55	1.54	−.41	4.23	.26	14.31	.13
114b	74.05	1.13		4.49		14.44	
115a	73.42	.74	−.19	3.93	.49	16.69	−.43
115b	73.55	.55		4.42		16.26	
116a	74.40	1.28	−.26	4.49	.26	15.43	−.10
116b	73.76	1.02		4.75		15.33	

In connection with the changes in reducing sugar the effect of cutting on the behavior of the potatoes must be considered. One of the most pronounced effects of wounding plant organs is a stimulation of respiration. The respiration of sweet potatoes is nearly doubled when they are split longitudinally, and the effect, though decreasing, extends over many days. By reason of this increased respiration split potatoes consume a much larger part of their reducing sugar than do whole potatoes. Nevertheless, in spite of this excessive respiration, there was, on the whole, during the first period a slight increase in reducing sugar, which is significant in comparison with the distinct loss during the second period. It appears clear, therefore, that more reducing sugar was formed during the first period than during the second; for during the first period the production of reducing sugar kept pace with its utilization, while during the second period the production was not sufficiently rapid to compensate for the quantity used.

Further evidence that more reducing sugar is formed in the potatoes during the first period than is indicated by the figures in the difference column is furnished by the whole potatoes stored with the first set of halves and split at the end of the first period. The percentage of reducing sugar in these "a" halves of the second period is much greater than in the "b" halves of the first period, with which they are comparable as to time of storage. Unfortunately, there is no such control for the behavior of the halves stored during the second period.

The potatoes used in the third experiment at 30° C. were allowed to remain in the ground for 15 days after the vines had been destroyed. They may therefore be considered to have been in "storage" in the ground during that period. The temperatures during that time, as given by observations of the United States Weather Bureau at Washington, D. C., were as follows:

Date.	Maximum.	Minimum.	Mean.	Date.	Maximum.	Minimum.	Mean.
	°C.	°C.	°C.		°C.	°C.	°C.
Oct. 22	13.3	1.6	7.8	Oct. 30	12.8	5.6	8.9
23	17.8	1.6	10.0	31	8.9	1.6	5.6
24	18.9	13.3	16.1	Nov. 1	11.1	0	5.6
25	19.4	13.3	16.7	2	12.8	.6	6.7
26	17.8	10.6	14.4	3	16.7	−1.1	7.8
27	20.6	6.1	13.3	4	17.2	7.2	12.2
28	22.8	10.6	16.7	5	14.4	1.1	7.8
29	17.8	6.1	12.2	6	15.6	−1.1	7.2

If the cutting of the vines has any effect on the carbohydrate transformations in the roots, the initial changes in these potatoes would have been inaugurated during the period after the vines had been cut and while the roots were still in the ground. However, the changes in these followed the same general course as those in the freshly dug potatoes.

There was a large loss of starch and a great accumulation of sugar during the first period, very little further loss of starch and accumulation of sugar during the second period, and a slight increase in reducing sugar during the first period, with a small loss during the second. But if the data of this experiment are compared with the corresponding data of the first and second experiments, it will be noted that the starch content of the sweet potatoes in the third experiment at the time they were dug is, on the whole, lower than that of the freshly dug potatoes in the first and the second experiments, and the cane sugar is higher, as though a part of the starch had already been converted at the time when the roots were dug. Furthermore, it will be noted that the loss of starch and the increment in cane sugar during the first period are a little less than in the corresponding periods of the first and second experiments. These facts show that as a result of the cutting of the vines the carbohydrate transformations had been initiated in these potatoes while they were still in the ground, but that the changes did not proceed as rapidly at the temperature of the soil as at 30°.

The results of the experiments at 30° C. may be summed up thus: In the freshly dug sweet potatoes whose vines were intact there was a large loss of starch and increase of cane sugar during the first period of 12 days, and very little further change in these substances during the second period. The changes in reducing sugar are obscured by the active respiration induced by high temperature and wounding, but, on the whole, the data show that there was a more extensive formation of reducing sugar during the first period than during the second. The potatoes which had been left in the ground for some time after the vines had been cut showed the same general phases of change, but their starch content was on the whole lower and their sugar content higher at the time of digging, and the rate of starch conversion during the first period was lower than in the potatoes dug while the vines were still intact. These conditions indicate that the carbohydrate transformations had proceeded to some extent in these potatoes after the vines had been cut and while the roots were still in the ground.

If the experiments at 5° C. (Table IV) are now examined, a marked contrast is found between these and the experiments at 30°. In the first two experiments with potatoes whose vines had remained active up to the time of digging, the loss of starch during the first period is much less than at 30°, but the loss continues at approximately the same rate during the second period. With respect to the behavior of the cane sugar the contrast between the potatoes at 30° and those at 5° is equally marked. At 5° there is only an insignificant increase in cane sugar during the first period, but a marked increase during the second. The reverse is true of the reducing sugar. There is a considerable accumulation during the first period and a marked reduction during the second.

TABLE IV.—*Changes in the composition of sweet potatoes at 5° C.*

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
33a	74.22	0.91	1.33	1.73	0.45	18.53	−1.89
33b	72.94	2.24		2.18		16.64	
34a	72.21	1.23	1.07	2.14	.35	20.29	−1.82
34b	72.20	2.30		2.49		18.47	
35a	71.28	1.27	.80	2.27	.38	20.45	−1.05
35b	69.22	2.07		2.65		19.40	
36a	71.02	1.37	.90	1.98	.79	20.92	−1.37
36b	70.71	2.27		2.77		19.55	
37a	73.99	1.40	1.00	2.07	.40	17.65	−1.06
37b	72.82	2.40		2.47		16.59	
38a	74.52	1.01	.71	2.18	.56	17.42	−1.16
38b	72.35	1.72		2.74		16.26	

FIRST EXPERIMENT (SECOND PERIOD)

39a	72.76	1.93	−0.34	2.62	1.89	18.00	−1.52
39b	70.71	1.59		4.51		16.48	
40a	73.46	2.19	−.21	2.88	1.62	16.94	−1.42
40b	72.40	1.98		4.50		15.52	
41a	73.74	2.17	−.12	3.06	1.66	16.69	−1.65
41b	72.37	2.05		4.72		15.04	
42a	72.59	1.79	−.13	3.18	1.16	17.76	−.90
42b	70.86	1.66		4.34		16.86	
43a	74.52	2.18	−.32	2.65	1.89	15.82	−1.45
43b	73.77	1.86		4.54		14.37	
44a	73.35	1.79	−.18	3.19	1.95	17.23	−2.17
44b	72.24	1.61		5.14		15.06	

SECOND EXPERIMENT (FIRST PERIOD)

82a	70.99	0.53	2.21	2.03	−0.12	21.26	−2.04
82b	72.61	2.74		1.91		19.22	
83a	76.07	1.14	1.04	2.06	−.04	16.41	−1.63
83b	75.93	2.18		2.02		14.78	
84a	72.38	.78	1.02	2.25	.48	20.37	−1.75
84b	72.51	1.80		2.73		18.62	
85a	73.49	.71	1.08	2.08	.21	19.37	−1.23
85b	72.53	1.79		2.29		18.14	
86a	73.47	.82	1.06	2.52	.28	18.94	−1.25
86b	72.11	1.88		2.80		17.69	
87a	73.99	.86	1.39	1.61	.34	19.25	−1.67
87b	72.87	2.25		1.95		17.58	

TABLE IV.—*Changes in the composition of sweet potatoes at 5° C.*—Continued

SECOND EXPERIMENT (SECOND PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
88a	71.16	1.90	−0.51	3.12	2.33	19.23	−1.61
88b	69.99	1.39		5.45		17.62	
90a	72.32	2.06	−.27	2.82	1.99	18.04	−1.42
90b	71.08	1.79		4.81		16.62	
91a	73.50	2.40	−.15	2.40	2.46	17.04	−2.55
91b	74.43	2.25		4.86		14.49	
92a	74.48	1.67	.25	2.91	2.30	15.72	−2.21
92b	75.24	1.92		5.21		13.51	
93a	70.65	2.04	−.18	3.40	1.99	19.48	−1.90
93b	71.43	1.86		5.39		17.58	
94a	73.26	2.08	−.20	3.66	1.93	16.97	−1.46
94b	73.16	1.88		5.59		15.51	
THIRD EXPERIMENT (FIRST PERIOD)							
125a	75.81	0.74	0.57	3.04	1.88	15.77	−2.38
125b	76.31	1.31		4.92		13.39	
126a	74.55	.98	.42	3.43	1.44	16.79	−1.96
126b	74.72	1.40		4.87		14.83	
127a	74.27	1.19	.25	3.48	1.72	16.89	−2.32
127b	74.52	1.44		5.20		14.57	
128a	74.87	1.05	.57	3.01	1.20	16.78	−1.92
128b	74.80	1.62		4.21		14.86	
129a	76.38	1.16	.18	3.32	1.18	14.62	−1.78
129b	76.00	1.34		4.50		12.84	
130a	72.65	.87	.35	3.62	1.25	18.69	−2.23
130b	73.69	1.22		4.87		16.46	
THIRD EXPERIMENT (SECOND PERIOD)							
131a	73.95	1.44	−0.19	4.67	2.67	14.67	−2.43
131b	72.90	1.25		7.34		12.24	
133a	76.15	1.49	.53	4.36	1.90	13.10	−2.59
133b	77.19	2.02		6.26		10.51	
134a	75.28	1.89	.22	4.26	2.12	13.94	−2.69
134b	76.32	2.11		6.38		11.25	
137a	73.85	1.58	.09	4.60	1.90	15.71	−2.54
137b	73.63	1.67		6.50		13.17	
138a	72.71	1.55	−.11	4.91	2.49	16.26	−2.59
138b	73.40	1.44		7.40		13.67	
139a	75.55	1.15	−.31	5.61	1.60	12.96	−1.30
139b	73.28	.84		7.21		11.66	

In the third experiment at 5°, which was carried out with potatoes that had been left in the ground for some time after the vines had been destroyed, the conversion of starch took place during both periods as in the other two experiments, but in contrast with these the accumulation of cane sugar took place not only in the second but also in the first period. At the same time there was a slight increase in reducing sugar during the first period and scarcely any further increase during the second. A

further fact should be noted—viz, that the starch content of these potatoes at the beginning of the first period is comparable in general with that of the potatoes at the beginning of the second period in the other experiments, while the final starch is much lower than in the other two groups. Similarly, the cane-sugar content at the beginning of the first period is comparable with that of the other groups at the beginning of the second period, but the final cane-sugar content is much higher than in either of those.

Here it is even more evident than in the corresponding experiment at 30° C. that the carbohydrate transformations were well under way at the time when the sweet potatoes were dug and that the data given in Table IV merely show the continuation of the processes which had already been started in the ground.

If the experiments at 5° are now summed up, it is found that whether the potatoes had been dug while the vines were still active or some time after the vines had been destroyed there was a fairly uniform loss of starch during both periods. In the first two experiments only inconsiderable quantities of cane sugar were formed during the first period, but during the second period there was a marked accumulation of cane sugar. In the third experiment the accumulation of cane sugar was marked during both periods. In contrast to the cane sugar, there was a considerable accumulation of reducing sugar during the first period in the first two experiments and a slight loss during the second period. In the third experiment there was little or no accumulation during either period.

The results of the experiments at 15.5° C. (Table V) do not present the same degree of uniformity as those at the other temperatures, but certain definite tendencies are evident. In the first experiment the loss of starch was large during the first period, but during the second the loss was not so great. Correspondingly, there was a considerable quantity of cane sugar formed during the first period and much less during the second. Very little change in the reducing sugar is evident during the first period, but during the second there is a distinct loss. It should be recalled here that the halves used in this experiment lost a large amount of water and that their behavior may have been influenced thereby, for from the work of Lundegårdh [1] it appears that the balance between oil and starch and sugar and starch in seedlings is shifted with changes in moisture content. The behavior of the roots in the second experiment is probably more nearly normal. Here the loss of starch is lower during the first period than at 30°, with no further loss during the second. The accumulation of cane sugar is not as great at first as at 30°, but is distinctly larger than during the second period. The increase in reducing sugar during the first period was comparable to that observed at 5°. During the second period there was a slight loss.

[1] Lundegårdh, Henrik. Einige Bedingungen der Bildung und Auflösung der Stärke. Ein Beitrag zu Theorie des Kohlehydratstoffwechsels. *In* Jahrb. Wiss. Bot., Bd. 53, Heft 3, p. 421–463. 1914.

TABLE V.—*Changes in composition of sweet potatoes at 15.5° C.*

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
10a	75.18	0.90	−0.24	2.30	2.75	17.17	−3.07
10b	63.44	.66		5.05		14.10	
11a	73.17	1.09	− .06	1.84	2.57	19.49	−2.97
11b	67.55	1.03		4.41		16.52	
12a	72.96	.82	0	2.88	3.14	19.08	−3.42
12b	62.87	.82		6.02		15.66	
30a	72.77	.75	− .07	2.09	2.69	19.63	−3.68
30b	62.20	.68		4.78		15.95	
31a	73.26	.94	− .25	2.01	2.73	19.25	−3.51
31b	67.32	.69		4.74		15.74	
32a	74.13	1.02	.04	2.44	2.04	17.96	−2.36
32b	65.64	1.06		4.48		15.60	

FIRST EXPERIMENT (SECOND PERIOD)

24a	70.10	1.52	−0.63	4.17	2.52	18.53	−2.06
24b	66.82	.89		6.69		16.47	
26a	71.85	1.16	− .58	5.12	.86	16.51	−1.29
26b	68.08	.58		5.98		15.22	
29a	71.64	1.48	− .60	3.57	1.94	18.26	−1.06
29b	66.18	.88		5.51		17.20	
51a	70.71	1.30	− .41	4.46	2.46	17.99	−1.85
51b	67.13	.89		6.92		16.14	
52a	69.12	1.36	− .33	5.02	1.12	19.52	−1.16
52b	66.61	1.03		6.14		18.36	
53a	71.51	1.44	− .68	4.52	1.91	17.89	−1.68
53b	67.74	.76		6.43		16.21	

SECOND EXPERIMENT (FIRST PERIOD)

55a	74.13	0.65	1.16	2.11	0.91	18.52	−1.78
55b	73.23	1.81		3.02		16.74	
56a	73.42	.82	1.08	2.66	.75	18.56	−1.48
56b	73.18	1.90		3.41		17.08	
57a	73.14	.85	.85	2.23	.87	19.19	−1.47
57b	71.95	1.70		3.10		17.72	
58a	73.88	.61	1.00	2.42	1.00	18.50	−1.89
58b	73.66	1.61		3.42		16.61	
59a	75.10	.83	.68	2.56	.91	17.23	−1.46
59b	73.66	1.51		3.47		15.77	
60a	75.61	.89	.72	2.13	.58	17.15	−1.18
60b	73.63	1.61		2.71		15.97	

TABLE V.—*Changes in composition of sweet potatoes at 15.5° C.*—Continued

SECOND EXPERIMENT (SECOND PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	Per cent.	*Per cent.*		*Per cent.*		*Per cent.*	
64a	73.29	1.05	0.03	3.30	0.36	17.07	0.10
64b	72.39	1.08		3.66		17.17	
65a	72.92	1.50	−.16	2.54	.69	17.91	−.34
65b	71.50	1.34		3.23		17.57	
66a	70.53	1.76	−.42	3.69	.57	19.24	.13
66b	68.75	1.34		4.26		19.37	
67a	73.34	1.39	−.10	3.31	.49	16.84	−.36
67b	72.49	1.29		3.80		16.48	
70a	71.72	1.39	−.06	2.91	.40	18.78	−.35
70b	70.85	1.33		3.31		18.43	
71a	73.30	1.55	−.21	3.30	.59	16.97	−.23
71b	71.72	1.34		3.89		16.74	

THIRD EXPERIMENT (FIRST PERIOD)

106a	75.20	0.77	0.68	2.81	−0.05	16.35	−0.22
106b	74.32	1.45		2.76		16.13	
107a	76.92	.72	.49	3.20	.15	14.95	−.66
107b	74.43	1.21		3.35		14.29	
108a	77.38	1.13	.60	2.59	.42	14.65	−.91
108b	75.58	1.73		3.01		13.74	
122a	75.60	.93	.55	2.92	.45	16.01	−1.18
122b	75.48	1.48		3.37		14.83	
123a	75.75	.48	.50	3.90	.12	15.00	−.72
123b	72.63	.98		4.02		14.28	
124a	74.54	.98	.55	3.00	.12	17.33	−1.07
124b	74.24	1.53		3.12		16.26	

THIRD EXPERIMENT (SECOND PERIOD)

118a	72.76	1.62	−0.01	2.95	0.25	18.26	−0.77
118b	72.95	1.61		3.20		17.49	
119a	74.93	1.61	−.20	3.36	.06	15.73	−.12
119b	74.48	1.41		3.42		15.61	
120a	75.16	1.74	−.13	3.56	−.13	15.21	.13
120b	74.11	1.61		3.43		15.34	
141a	75.21	2.00	−.17	3.86	−.08	15.08	0
141b	74.53	1.83		3.78		15.08	
142a	73.73	1.48	.33	3.54	.64	16.42	−1.33
142b	75.14	1.81		4.18		15.09	
143a	75.26	1.68	−.15	3.26	−.04	15.24	.02
143b	75.57	1.53		3.22		15.26	

In the sweet potatoes which had already undergone a period of "storage" in the ground there was on the whole very little further loss of starch and practically no further accumulation of cane sugar. The reducing sugar shows distinct increase during the first period and a loss during the second.

DISCUSSION OF DATA

If the results of these experiments are considered in a general way, it is found that the rate of starch conversion varies with the temperature. At 30° C. the process is rapid at first, but soon appears to approach a point where no further conversion takes place. At 15.5°, if the second experiment is regarded as typical, the rate of starch hydrolysis is less rapid, but at this temperature also the process seems to approach a state of completion. At 5° the process is distinctly retarded, but it continues without decrease during the period covered by the experiments.

The rate of accumulation of cane sugar also varies with the temperature. At 30° the greater part of the cane sugar is formed during the first 10 to 12 days after the roots have been severed from the vines, but the rate of accumulation diminishes rapidly. At 5° very little cane sugar is produced during the first 10 to 12 days, but subsequently the rate of accumulation is considerably increased, as if there were a lag at first in the formation of cane sugar at this temperature.

The behavior of the reducing sugar is obscured by its utilization in respiration. It is nevertheless evident from the data presented in this paper and in former papers that at 30° C. the production of reducing sugar is sufficiently rapid to provide all that is used in respiration and still permit a considerable accumulation which, under normal conditions, is not far behind that at 5°. At 15.5° (second experiment) and at 5° there is a marked accumulation of reducing sugar at first, but at these temperatures, as well as at 30°, there is very little further accumulation, or even a slight subsequent loss.

The apparent lag at first in the accumulation of cane sugar associated with the marked accumulation of reducing sugar at low temperatures may throw some light on the process of the formation of cane sugar from starch. In the experiments at 5° C. reducing sugar was obviously formed during the first period as a result of the conversion of starch. The disappearance of starch continued at the same rate during the second period. During this period there was, however, no further increase in reducing sugar, but a large increase in cane sugar. Since it is not likely that in the one instance reducing sugar resulted directly from the conversion of starch, and in the other, cane sugar, it may be assumed that the production of reducing sugar went on at a rate corresponding to the loss of starch during both periods and that the excess which was produced during the second period was utilized in the formation of cane sugar. In this connection it is worthy of note that the concentration of reducing sugar always remains comparatively low. Even at low temperatures, at which starch transformation goes on continuously and respiration is reduced to a minimum, the reducing sugar content does not rise above 2 to 2.5 per cent. It appears, therefore, that with the exception of the quantity used for respiration the reducing sugar is transformed into cane sugar as fast as it is formed from starch. Its rate of transformation

would, therefore, be correlated with that of the starch. From these considerations it appears that the hydrolysis of starch in the sweet potato results directly in the formation of reducing sugar, as has been observed in cotyledons and other living plant organs, and that the cane sugar is synthesized from the reducing sugar. Cane sugar is therefore the end product of this series of carbohydrate transformations.

It has sometimes appeared from the extensive accumulation of cane sugar in plant organs at low temperatures that this process went on more rapidly at low than at high temperatures. Such a conclusion would seem to be justified if later phases of the process were compared at different temperatures, as illustrated by the data relating to the second periods of the experiments at 30° and at 5°. These data show that during these periods the loss of starch and the gain in sugar was greater at 5° than at 30°. On the basis of the interpretation given above, however, it is clear that all these reactions conform in general to the Van't Hoff temperature rule regarding chemical reactions. Thus, the rate of conversion of starch is higher at 30° than at 5°, but the reaction obviously approaches an end point which is more rapidly approximated at 30° than at 5°; hence, the reaction slows down more rapidly at 30° than at 5°. It is evident also that the production of cane sugar is more rapid at higher temperatures, and that the reaction, which is prolonged at 5°, nears an end point more quickly at 30°. Hence, if these reactions at different temperatures are compared in their later phases, they will appear to be more rapid at the lower temperature. In the common Irish potato, as well as in some other living plant organs, the series of reactions resulting in the production of cane sugar from starch has been found to be reversible. It is not unlikely that in the sweet potato also the reaction is reversible and that thus the attainment of a final equilibrium between the starch, reducing sugar, and cane sugar is explained. The end point of the reaction or the point of equilibrium is greatly shifted with change of temperature, with the effect that at low temperatures the system permits a greater concentration of sugar than at higher temperatures.

On the basis of these considerations a rational interpretation can be given of the rapid initial carbohydrate transformations, which have been mentioned several times and which it was in part the object of this work to study more fully. The fact that there is a comparatively rapid transformation of starch to cane sugar in sweet potatoes during the first few days after they have been dug and a very much slower transformation subsequently is supported by the data of the experiments conducted at 30° and at 15.5°. At 5°, however, the disappearance of starch continues at about the same rate during both periods, while the rate of accumulation of cane sugar is low at first and higher afterwards. All these facts are explicable by the interpretation given above. We have to do here with processes whose rate depends on the temperature and which at

higher temperatures approach an end point very rapidly, so that we find at first a rapid transformation and after a few days almost a cessation of the processes. At 5° the rates of the reactions are greately reduced, but the processes continue over a much longer period of time, and the starch conversion and sugar accumulation are much more extensive. At this temperature the course of the reactions becomes clear. The conversion of starch results in the formation of reducing sugar. As the concentration of reducing sugar increases, the rate of formation of cane sugar rises, but at first there is a lag in the production of cane sugar.

There remains to be considered the influence of the vines on the carbohydrate transformations of the sweet potato. From work formerly reported it appears that the conversion of starch to sugar does not take place to any marked extent in the growing potato, and that the inauguration of this process is probably associated with the cessation of the flow of materials from the vines. The data of the third series of experiments confirm this suggestion and show that when the vines are destroyed, even if the roots are left untouched in the ground, the carbohydrate transformations begin. In the third series of experiments carried out with sweet potatoes which were left in the ground for some time after their vines had been cut, there is evidence which has been set forth in the description of the experiments that the carbohydrate transformations were well under way when the potatoes were dug. It is therefore safe to conclude that the activity of the vines inhibits the conversion of starch to sugar in the growing sweet potato.

CONCLUSIONS

From the data given in this paper it appears that in the carbohydrate transformations in stored sweet potatoes starch is first converted to reducing sugar and cane sugar is synthesized from the reducing sugar. The rates of starch hydrolysis and of sugar synthesis in a general way conform to the Van't Hoff temperature rule for rates of chemical reactions. At high temperatures the reactions are rapid at first, but soon become slower and approach an end point. At low temperatures the rates are slower and the end point is so shifted as to permit a greater concentration of sugar. The reactions are continuous.

In the growing sweet potato the concentration of sugar remains comparatively low. The extensive conversion of starch into sugar appears to be inhibited by the activity of the vines. When the vines are destroyed and the flow of materials to the roots is thus interrupted, the carbohydrate transformations characteristic of stored sweet potatoes are begun, even if the roots are left in the ground.

DIURESIS AND MILK FLOW

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INTRODUCTION

In studying the comparative efficiency of the nitrogen of alfalfa hay (*Medicago sativa*) and corn grain (*Zea mays*) for milk production, data were accumulated and published from this laboratory which suggested that alfalfa hay when fed in large amounts often acts as a diuretic and thus depresses the volume of milk flow.[1] While this relation was not found with all the experimental animals (cows), it was, nevertheless, deemed of sufficient importance to merit the study of the influence of specific diuretics on milk flow, as it was barely possible that the diuresis which was produced upon the feeding of alfalfa hay was not in itself responsible for the depression of mammary activity.

In view of the importance which hitherto unknown constituents of diets and rations have lately assumed, it is of the greatest interest to dissect the various factors normally operative in the animal body when feeding any of our ordinary rations. Dairy chemists have spent much time and effort in studying the various factors which influence the secretion of milk and its composition. It seemed not improbable that if any of the well-known diuretics were able to influence milk secretion the means to vary the proportion of individual constituents might also be at hand.

EXPERIMENTS WITH DIURETICS ON GOATS

Two goats in full milk flow were used as the experimental animals. They were individually confined in metabolism cages which made possible the separate quantitative collection of urine and feces. They were fed and milked twice a day, the milk of two consecutive milkings being composited for analysis and measurement of volume. Careful measure of the water consumed and urine voided was recorded. Control of the ration consumed was kept only to the extent that results obtained could not possibly be due to variation in food intake. Goat 1, weighing 95 pounds, was fed daily a ration consisting of 2 pounds of oats (*Avena sativa*), 0.5 pound of June-grass hay (*Poa pratensis*), 60 gm. of air-dried casein, 1 pound of fresh sugar beets (*Beta vulgaris*), and 2 gm. of common salt (sodium chlorid). This provided sufficient energy and a suffi-

[1] Hart, E. B., Humphrey, G. C., Willaman, J. J., and Lamb, A. R. The comparative efficiency for milk production of the nitrogen of alfalfa hay and the corn grain. Preliminary observations on the effect of diuresis on milk secretion. *In* Jour. Biol. Chem., v. 19, no. 1, p. 127–140. 1914.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 15
Dec. 27, 1915
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ciently narrow nutritive ratio to serve excellently for milk production. Goat 2, weighing 81 pounds, was fed from 1.5 to 2 pounds of oats, 0.5 to 0.75 pound of June-grass hay, and 1 gm. of common salt daily, though the latter was often refused. Great care was taken that any variations in salt intake were not of sufficient moment to influence the character of the results obtained. Data obtained during periods of low consumption or of unusual restlessness of the animal were discarded, as such conditions obviously disturb the milk secretion. Everything possible was done to contribute to the comfort of the experimental animal, in accordance with good dairy practice.

First, it was desired to ascertain if specific diuretics were able at all to influence the volume of milk secreted. At the same time in some instances determinations of the total solids and nitrogen in the milk were made. As it was suggested in the publication referred to that the salts of the alfalfa ration might have been responsible for the diuresis, sodium acetate was the diuretic selected for the first trials. It was given per os to goat 1, at first with her drinking water, but later, as larger amounts were given, as a drench immediately after each milking. During a 4-day period, when there were administered, respectively, 20, 20, 50, and 50 gm. of sodium acetate daily, no diuresis resulted and no change in the milk volume occurred. It was not until the dose was increased to 80 gm. that the milk flow was materially affected, but even here, as seen in Table I, the diuresis was not pronounced.

TABLE I.—*Effect of sodium acetate on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Nitrogen in 5 c. c.	Remarks.
	C. c.	*C. c.*	*C. c.*	*Per cent.*	*Mgm.*	
Nov. 24.....	2,950	1,150	860	15.89	34.7	No additions to ration.
25.....	2,000	750	830	16.40	35.7	Do.
26.....	3,000	1,250	880	15.84	31.5	Do.
27[a].....	2,700	1,250	610	18.22	33.7	Collection from 80 gm. of sodium acetate.
28[b].....	2,000	1,400	660	17.89	31.5	Collection from 100 gm. of sodium acetate.
29[c].....	1,725	450	925	14.34	30.0	No additions to ration.
30[d].....	2,950	635	930	15.25	31.4	Do.
Dec. 1.....	1,670	760	985	15.39	34.0	Do.

[a] Small amount of the casein beet mixture not consumed.
[b] No casein or beets consumed.
[c] No casein or beets given; 12 ounces oats left unconsumed.
[d] No casein given.

An increase in percentage of the solids in milk with the decrease in volume is pronounced, while the nitrogen content is unaffected. (See p. 566.) While the indications from the data on the administration of sodium acetate are that the volume of milk flow is decreased with diuresis, yet with the administration of such large amounts of the salt as was found necessary, too severe disturbances of the appetite resulted

to make the data serve their purpose. Furthermore, the urine was so strongly alkaline in reaction that from all appearances of the vulva a marked irritation of the urinary tract had resulted. Obviously sodium acetate was not a good diuretic to use for the solution of the problem at hand. Later some success was obtained with the use of sodium citrate, which with goat 2 upon the administration of 40 gm. in two portions increased the urinary volume from 170 to 550 c. c. and decreased the flow of milk from a volume of 395 to 350 c. c. Its use was not continued.

It was suggested that with the now well-known diuretic properties of the methyl purins, theocin might be a suitable agent. It was administered per os to goat 1 in gelatin capsules in two doses daily during a 9-day period, during which the daily dose was gradually increased to 600 mgm. Inasmuch as the dose for man ordinarily is given at 200 to 400 mgm., it must have been large enough; yet at no time was a diuretic effect noticed. Whether this is due to the difficulty of absorption with the ruminant was not determined, but at any rate during rumination the bitter taste of the regurgitated theocin destroyed the appetite of the animal to such an extent that even if it should have been effective in larger doses its continued administration was out of the question.

Urea was used next and with good results when given in large doses, as shown in Table II.

TABLE II.—*Effect of urea on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Remarks.
	C. c.	*C. c.*	*C. c.*	
Dec. 19	2,000	685	790	No additions to ration.
20	2,000	625	770	Do.
21[a]	2,675	1,925	500	Collection from 50 gm. of urea.
22	2,700	600	850	No additions to ration.
23	2,000	700	930	Do.
24	3,000	2,060	660	Collection from 30 gm. of urea.
25	2,900	1,160	855	No additions to ration.
26	2,525	1,260	780	Do.
27	3,285	1,825	640	Do.
28	2,450	850	786	Do.
29	1,000	460	700	Do.
30	2,575	500	755	Do.
31	1,925	625	740	Do.

[a] Little casein consumed; its feeding was discontinued from here on.

Urea when given in diuretic doses decreases the volume of milk secreted from 18 to 35 per cent, as seen in Table II. The diuresis in each case is followed by a period of one day in which the daily consumption of water is higher than normal, which suggests that the decreased flow of milk is caused by the withdrawal of body fluids from the mammary gland in an attempt of the animal to free its system of the diuretic. Compensation evidently is not immediately effected by the imbibition of sufficient water, and the body secretions are made to suffer as the result.

An attempt was made to accentuate the effect of the diuretic on the milk flow by keeping the water intake at a level which under normal conditions would be entirely sufficient for the animal, but with the additional requirements during diuresis draw heavily upon the body fluids. Goat 1 was used as the experimental animal. See Table III.

TABLE III.—*Effect of urea with constant level of water intake on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	C. c.	*C. c.*	*C. c.*	*Per cent.*	*Pr. ct.*	*Mgm.*	
Jan. 26..	2,000	715	780	15.06	5.5	33.9	No additions to ration.
27..	2,000	640	840	15.06	5.4	33.2	Do.
28..	2,000	815	695	16.19	6.7	33.0	Collection from 30 gm. of urea.
29..	2,000	550	860	15.70	6.7	29.6	Do.
30..	2,000	675	900	15.06	5.5	32.8	Do.
31[a]..	2,000	260	845	15.99	5.9	33.6	No additions to ration.
Feb. 1..	2,000	855	725	16.18	5.9	35.7	Do.
2..	2,000	350	820	15.08	5.7	35.1	Do.

[a] Animal very restless; beets not all consumed.

As seen in Table III, renal activity after the effects of the first day was not sufficient to draw noticeably on the mammary secretion for fluids. At no time when urea was given, even in the above experiment, did the animal show any abnormal desire for water; in fact, the water supply when replenished in the morning was usually left untouched for some time. Yet it is hardly to be questioned that the animal was in great need of water. On the morning of February 2, after the previous day's collection had been made, 25 gm. of urea were given in one dose to determine whether larger quantities of urea were necessary to produce the desired results. This amount of urea, while large, would not furnish any more urea for excretion through the kidneys than 70 gm. of protein, and no untoward effects were expected. Yet five minutes after the urea was given the animal lay down and soon passed into violent convulsions, which terminated fatally in 1 hour and 15 minutes. A morphine hypodermic was of no avail in preventing death. A post-mortem examination gave no clue to the cause of death. Apparently the maximum quantity of urea which could possibly be retained with safety in the circulation had accumulated during the previous period of urea administration. With the sudden flooding of the system with the additional 25 gm. of urea the safety limits were exceeded and death resulted. Immediately previous to the administration of the final dose of urea the animal was ruminating and apparently normal in all respects. The urea used was a Kahlbaum preparation and undoubtedly was free from such other toxic compounds as cyanid or cyanate, as no untoward results followed the subsequent use of urea from the same reagent bottle. It was barely

possible that the previous severe régime of sodium acetate and purin feeding may have injured the kidneys sufficiently to account for the results obtained.

In other trials it was repeatedly demonstrated that the administration of urea upon consecutive days would not continue to influence milk secretion even though diuresis obtained. This is brought out in Table IV.

TABLE IV.—*Effect of repeated urea administration on the milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	C. c.	*C. c.*	*C. c.*	*Per ct.*	*Per ct.*	*Mgm.*	
Jan. 15...	1,850	1,135	880	15.00	5.6	33.5	No additions to ration.
16...	2,500	975	840	14.85	5.0	33.5	Do.
17...	2,850	1,125	840	14.56	5.4	34.3	Do.
18...	3,000	1,685	710	16.55	6.5	35.1	Collection from 30 gm. of urea.
19...	3,550	2,100	850	15.72	6.4	32.3	Do.
20...	4,000	2,410	780	16.12	6.8	32.2	Do.
21...	3,000	1,625	845	15.85	6.15	31.6	Do.
22...	3,000	735	965	15.10	5.7	31.7	No additions to ration.
23...	4,100	345	850	15.07	5.4	33.5	Do.
24...	2,075	525	875	15.00	5.25	33.9	Do.

It is significant that the consumption of water upon repeated administrations of urea increases with the diuresis. Whatever factors may be responsible for the symptoms of increased thirst when urea is given, they do not become operative until the water supply of the body is drawn upon so heavily that milk secretion is reduced. The stimulation of the mechanism for maintaining the concentration of the body fluids normal is then sufficient to cause the animal to imbibe enough water for all its excretory and secretory processes.

In this connection it was of great interest to determine the effect of the administration of sodium chlorid upon milk secretion. Table V gives the data obtained with goat 2.

TABLE V.—*Effect of sodium chlorid on the milk flow of goat 2*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	C. c.	*C. c.*	*C. c.*	*Per ct.*	*Per ct.*	*Mgm.*	
July 19...	1,500	190	410	16.13	6.8	30.4	No additions to ration.
20...	1,050	155	390	16.25	6.5	30.4	Do.
21...	2,800	355	420	15.58	6.2	30.3	Collection from 20 gm. of sodium chlorid.
22...	1,175	355	400	15.91	6.6	30.3	No additions to ration.
23...	1,475	160	375	15.44	6.7	29.2	Do.
24...	2,425	580	410	14.59	5.5	28.8	Collection from 20 gm. of sodium chlorid.
25...	1,050	330	415	14.45	5.7	27.9	No additions to ration.
26...	800	170	395	14.77	5.7	29.0	Do.

While diuresis resulted and more water was lost through the gut, as indicated by a softer consistency of the feces, the volume of milk secreted was not decreased. This is to be explained by the fact that simultaneously with the increased urine flow more than sufficient water was consumed to cover the loss. By stimulation of thirst the excessive concentration of the body fluids was prevented, and the milk flow was not decreased.

In just what manner the relations between milk flow and urinary secretion with alfalfa hay are brought about is not clear. Whatever agent may be responsible for the diuresis, its action evidently is different from that of urea or sodium chlorid as observed in these studies with the goat.

INFLUENCE OF DIURESIS UPON THE COMPOSITION OF MILK

It will be noticed in Tables I and VI that with decrease of milk volume as caused by diuresis the percentage of total solids is increased. This increase is usually completely accounted for by the increase in fat content. The nitrogen content is not changed.

TABLE VI.—*Effect of diuresis on milk solids of goat 2*

PERIOD 1

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen per 5 c. c.	Remarks.
	C. c.	*C. c.*	*C. c.*	*Per ct.*	*Per ct.*	*Mgm.*	
May 21...	1,900	40	740	16.36	6.2	33.0	No additions to ration.
22...	1,800	140	705	16.38	6.4	32.7	Do.
23...	850	350	550	18.67	8.3	35.0	Collection from 20 gm. of urea.
24...	1,700	560	675	16.09	7.9	33.0	No additions to ration.
25...	1,570	100	640	16.89	6.9	33.9	Do.
26...	1,340	80	690	16.11	6.2	33.0	Do.
27...	1,490	350	600	16.83	6.9	33.0	Collection from 20 gm. of urea.
28...	1,300	110	650	16.45	6.8	30.9	No additions to ration.

PERIOD 2

June 1...	1,580	115	635	15.11	5.2	31.9	No additions to ration.
2...	1,830	140	620	15.30	5.4	30.3	Do.
3...	1,275	90	600	15.34	5.8	30.5	Do.
4...	1,800	380	540	15.91	6.3	30.4	Collection from 20 gm. of urea.
5...	1,125	145	600	15.58	5.9	29.5	No additions to ration.
6...	1,790	50	600	15.28	5.4	29.4	Do.
7...	1,850	365	550	15.86	6.4	29.6	Collection from 20 to 25 gm. of urea.
8...	1,940	220	640	15.31	6.0	27.5	No additions to ration.
9...	1,530	130	645	15.43	6.0	29.2	Do.
10...	1,450	130	655	15.05	5.8	29.8	Do.
11...	1,730	195	620	14.11	5.2	28.8	Do.

The constancy of the nitrogen content of the milk made it impossible that any of the administered urea found its way into the milk, which hypothesis was borne out by direct determination of urea in the milk.

One hundred c. c. of milk were measured off into a 250 c. c. volumetric flask, diluted with 100 c. c. of water, and the proteins removed at boiling temperature by the cautious addition of a 10 per cent solution of acetic acid. Generally about 1 c. c. was required. After cooling, the contents were made up to volume, set aside for 10 minutes, and then filtered through a dry folded filter. One hundred c. c. of the filtrate were pipetted off into an aeration bottle made slightly alkaline to phenolphthalein with a 10 per cent solution of sodium hydroxid and then acidified by the addition, drop by drop, of a 10 per cent solution of monobasic potassium phosphate (KH_2PO_4). After incubation for two hours at 41° C. with 2 c. c. of a 10 per cent solution of urease in the presence of toluol, the ammonia was aspirated into *N/28* hydrochloric acid. Fusel oil was used to prevent foaming. The air current was broken up into fine bubbles in the acid by firmly inserting a small plug of glass wool into the end of the tube dipping into the acid. Later, it was found feasible to make the urea determination without the previous removal of the milk proteins, as the fusel oil was sufficiently active in preventing foaming. A small amount of ammonia was found to be present in milk, but as this is practically negligible, the results are expressed as total ammonia in terms of milligrams of nitrogen per 100 c. c. of milk. (See Table VIII.)

TABLE VIII.—*Effect of the administration of urea to goat 2 on the urea content of milk*

Date.	Urea given.	Nitrogen as NH_3 and urea per 100 c. c. of milk.	Urine.	Milk.	Date.	Urea given.	Nitrogen as NH_3 and urea per 100 c. c. of milk.	Urine.	Milk.
	Gm.	*Mgm.*	*C. c.*	*C. c.*		*Gm.*	*Mgm.*	*C. c.*	*C. c.*
July 10...	0	13. 2	190	500	July 16...	20	11. 3	580	445
11...	0	10. 5	210	510	17...	20	7. 5	360	420
12...	0	13. 0	80	480	18...	20	10. 2	390	415
13...	0	9. 0	315	435	19...	0	7. 7	190	410
14...	0	9. 1	200	480	20...	0	11. 4	155	390
15...	20	11. 1	345	375					

The independence of the urea excreted and the urea put out in the mammary secretion strongly suggests that the urea in milk in large part is the result of mammary activity and not the result of a mere diffusion from the circulation.

CONCLUSIONS

(1) Urea administered in a diuretic dose is able to decrease temporarily the flow of milk. Upon repeated administration the increased intake of water which follows the impoverishment of the tissues with respect to

water content balances the draft for water imposed by the diuretic, and the milk secretion comes back to normal.

(2) Sodium chlorid with its diuretic action as well as its laxative effect is unable to depress milk secretion under normal conditions, as it simultaneously calls forth an excessive thirst, which increases the water intake.

(3) With the decreased flow of milk caused by a diuretic the percentage of solids is increased. Fat here is the principal variable.

(4) The mammary gland shows no tendency to absorb and subsequently put out in its secretion additional urea absorbed by the circulation.

(5) It is difficult to interpret the results sometimes obtained with alfalfa hay as due to diuresis alone if urea diuresis can be taken as a type.

PETROGRAPHY OF SOME NORTH CAROLINA SOILS AND ITS RELATION TO THEIR FERTILIZER REQUIREMENTS

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INTRODUCTION

In connection with the detail study of the soils of North Carolina, the writer has had occasion to make many mineralogical analyses of the existing soil types as defined by the United States Bureau of Soils. These examinations have included all types of any prominence thus far encountered in the survey and give some rather interesting data as to the formation and character of these soils which may be of more than local interest.

The available data showing the mineral composition of soils are meager. The scope of those found is so broad that definite conclusions can hardly be drawn as to the relationships which exist between the mineral component and the character of soils. The behavior of the various soil-forming minerals toward the forces of weathering will have to be known before the soil investigator will be able to solve many of the complex problems confronting him.

The methods used in these analyses are essentially those compiled by McCaughey and Frye.[1] Unfortunately, one serious criticism may be made regarding these methods—i. e., the defiance of members of the clay group against identification. It is quite possible that this group plays the most important rôle in the various soil phenomena of all the separates which compose the soil. Yet it would seem that since the clay owes its origin to the coarser particles, some definite knowledge of the composition of the latter would be imperative.

SOILS OF NORTH CAROLINA

The soils of North Carolina are quite heterogenous and furnish well-defined examples for a discussion of the petrography of soils. The State is divided into three provinces, determined largely by the physiographic provinces used in any study of physical geography. There are the old Appalachian, locally known as the Mountain section, Piedmont Plateau, and Atlantic Coastal Plain. As will be shown later, wide variations in the mineralogical composition of the soils of these provinces are encountered.

Practically all of the soils of the mountains are of residual origin and are derived from igneous and metamorphic rocks, mainly gneiss, schists,

[1] McCaughey, W. J., and Fry, W. H. The microscopic determination of soil-forming minerals. U. S. Dept. Agr. Bur. Soils Bul. 91, 100 p., 12 fig., 12 tab. 1913. Bibliography, p. 99–100.

and granites. The sandy loams, sands, and most of the loams are products of the gneiss and granites; the heavier loams, clay loams, and clays have been derived, for the most part, from schists.

With few exceptions, the soils of the Piedmont Plateau are residual. The rocks of this section are varied and complex, being composed of (1) such igneous material as diorite, diabase, gabbro, and granites; (2) such metamorphosed igneous material as gneiss, schists, and slate, and (3) such young sedimentary rocks as Triassic sandstone and shale.

None of the soils of the Atlantic Coastal Plain are residual. They all belong to the broad division known as "transported" and are composed of unconsolidated material laid down from the provinces of higher topography. Because of the abrasive and leaching forces which have entered into their formation, the least resistant minerals have been removed, quartz composing mainly the entire soil mass.

In the mineralogical composition of the soils series here reported, the average analyses of five samples of each series were taken. These samples were selected from widely separated areas in order that the series might be as nearly representative as possible. It was recognized at the outset that it would have been better to show the composition of the various types of a series, but space would not permit such procedure. However, it may be said as a general rule that there are no appreciable differences in the occurrence of the minerals in the various types of a series. There are wide variations in the preponderance of different minerals in the types, but usually each series carries the same minerals in all of its types.

To obtain these results, a separation by mechanical analyses of the sand, silt, and clay of each sample was necessary, and the mineral composition of the sand and coarse silt was determined. The clay particles were discarded as being too small for identification. The results are given in Table I, and include the estimation of all the minerals except quartz—the more abundant or characteristic minerals and the less abundant or secondary in quantity present.

A careful study of Table I will show some rather interesting data concerning the mineral component of the sand and silt particles of these soils. One of the most striking points is the wide difference in mineral complexity between soils of the Appalachian Mountains and those of the Piedmont Plateau and the Atlantic Coastal Plain. The soils of the Porters series are the predominating soils of the former province. The Toxaway soils, which are found in the valleys, are of alluvial origin modified by colluvial wash. In these soils there is a more decided occurrence of the original minerals of the parent rock than is found elsewhere.

TABLE I.—*Mineralogical composition of soils of North Carolina*

APPALACHIAN MOUNTAINS

Series.	Depth.	Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals in—		Remarks.
		Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
Porters (surface soil).	*Inches.* 0 to 8	52	60	Muscovite, biotite, epidote, orthoclase.	Biotite, muscovite.	Epidote, microcline, hornblende, magnetite, chlorite, tourmaline, rutile, zircon, sillimanite, serpentine, pyroxene, plagioclase, apatite.	Orthoclase, chlorite, microcline, epidote, sillimanite, rutile, zircon, hornblende, magnetite.	Soil characterized by high content of minerals not quartz, of which biotite and muscovite compose about 20 per cent.
Porters (subsoil)...	8 to 30	57	66	Biotite, muscovite, orthoclase, epidote.	.....do.....	Microcline, hornblende, magnetite, tourmaline, rutile, zircon, serpentine, pyroxene, plagioclase, apatite.	Hornblende, magnetite, chlorite, rutile, zircon, plagioclase, apatite.	As in the soil, the mica content is very high. Biotite is principally fresh; this also applies to orthoclase. Quartz is mainly primary.
Toxaway (surface soil).	0 to 11	50	57	Biotite, orthoclase, epidote, microcline, muscovite.	.....do.....	Hornblende, magnetite, chlorite, sillimanite, rutile, zircon, plagioclase, pyroxene, apatite.	Chlorite, magnetite, rutile, zircon, sillimanite, microcline, epidote, tourmaline.	Very high content of potash-bearing minerals. Biotite and orthoclase compose over 30 per cent. Both occur as fresh and altered fragments. Quartz grains somewhat rounded.
Toxaway (subsoil).	11 to 30	44	49	Biotite, orthoclase, epidote.	Biotite (altered and fresh), muscovite.	Hornblende, chlorite, magnetite, sillimanite, rutile, zircon, plagioclase.	Chlorite, magnetite, zircon, sillimanite, microcline, epidote, tourmaline.	High content of micas. Biotite at times shows deep-seated chemical alteration. Some secondary quartz present.

TABLE I.— *Mineralogical composition of soils of North Carolina*—Continued

PIEDMONT PLATEAU

Series.	Depth.	Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals in—		Remarks.
		Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
	Inches.							
Cecil (surface soil)..	0 to 8	30	34	Orthoclase, muscovite, biotite (altered and fresh), epidote, microcline.	Muscovite, biotite, orthoclase, epidote, microcline.	Hornblende, magnetite, rutile, zircon, chlorite, sillimanite, serpentine, garnet, plagioclase, apatite.	Hornblende, chlorite, sillimanite, rutile, zircon, magnetite, garnet.	Rather high content of minerals not quartz. Alteration has taken place among some of the minerals. Plagioclase and apatite found only as traces. Much secondary quartz.
Cecil (subsoil).....	8 to 36	28	33	Orthoclase, muscovite, biotite, epidote.	Muscovite, orthoclase, microcline, epidote.	Hornblende, chlorite, magnetite, zircon, rutile, sillimanite, garnet, serpentine, microcline, tourmaline, plagioclase, apatite.	Hornblende, chlorite, sillimanite, rutile, zircon, magnetite, plagioclase, apatite included in quartz.	Potash-bearing minerals most abundant. Minerals are for the most part altered. Much quartz-carrying infiltrated iron oxid.
Iredell (surface soil).	0 to 7	80	Very high.	Hornblende, epidote, augite, biotite.	Hornblende, epidote, biotite.	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline, apatite (free and included in epidote).	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline.	Quartz is a subordinate mineral in this series, it being less than 5 per cent in the silt. Potash feldspars occur as mere traces.
Iredell (subsoil)....	7 to 30	84	Very high.	Hornblende, epidote, augite, biotite.	Biotite, hornblende, epidote.	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline, apatite (free and included in other minerals).	Chlorite, muscovite, magnetite, plagioclase.	Quartz is even less than in surface soil. Biotite has been altered to a lesser degree than found in the surface. Quartz is mainly primary.
Granville (surface soil).	0 to 10	35	40	Microcline, orthoclase.	Microcline, orthoclase.	Epidote, hornblende, magnetite, zircon, rutile, tourmaline, biotite, plagioclase, sillimanite.	Hornblende, epidote, biotite, magnetite.	Potash feldspars compose the greater part of minerals not quartz. Biotite shows deep-seated chemical alteration.
Granville (subsoil).	10 to 28	30	38	.....do.....	Microline, ortnoclase, muscovite.	Hornblende, epidote, magnetite, rutile, zircon, tourmaline, sillimanite, plagioclase.	Hornblende, epidote, magnetite, rutile, zircon.	Potash feldspars as high as 22 per cent of minerals not quartz. Generally more in the silt than found in the surface. Some secondary quartz.

Georgeville (surface soil).	0 to 6	12	16	Orthoclase.....	Orthoclase, muscovite.	Magnetite, sillimanite, epidote, hornblende, chlorite, rutile, zircon, biotite, tourmaline, magnetite, microcline.	Epidote, sillimanite, tourmaline, chlorite, rutile, zircon, biotite.	Much quartz-carrying coatings of iron oxid. Minerals are of a refractory nature. Biotite and orthoclase are badly altered.
Georgeville (subsoil).	6 to 26	14	16	.....do.....	Orthoclase, microcline, muscovite.	Epidote, sillimanite, hornblende, rutile, zircon, microcline, magnetite, tourmaline.	Epidote, tourmaline, sillimanite, chlorite, biotite, rutile, zircon.	Very much the same mineralogical composition as found in the surface soil.
ATLANTIC COASTAL PLAIN								
Norfolk (surface soil).	0 to 7	2 to 4	6 to 9	None.....	None.....	Orthoclase (residues), microcline.	Epidote, tourmaline, zircon, rutile, magnetite, sillimanite, hornblende, muscovite, biotite, garnet.	Soil characterized by low content of minerals not quartz. Those existing are of a decidedly refractory nature.
Norfolk (subsoil)...	7 to 28	5	9	.....do.....	.....do.....	Orthoclase residues....	Epidote, tourmaline, zircon, rutile, magnetite, sillimanite, microcline, muscovite, chlorite, garnet, biotite.	Little difference between soil and subsoil in mineralogical complexity.
Portsmouth (surface soil).	0 to 6	1 to 3	4	.....do.....	.....do.....	Microcline, tourmaline, sponge spicules, zircon, rutile, hornblende, magnetite, muscovite, chlorite, garnet.	Sponge spicules, zircon, rutile, magnetite, hornblende, muscovite.	Only the highly refractory minerals and sponge residues present.
Portsmouth (subsoil).	6 to 26	2	5	.....do.....	.....do.....	Sponge spicules, microcline, orthoclase (badly weathered), muscovite, chlorite, magnetite, hornblende, zircon, epidote, tourmaline, garnet.	Weathered orthoclase, epidote, hornblende, rutile, zircon, muscovite, magnetite, sponge spicules.	Very much the same condition as found in the surface soil.
Orangeburg (surface soil).	0 to 8	6	7	.....do.....	Microcline.....	Orthoclase, microcline, epidote, hornblende, magnetite, zircon, rutile, tourmaline, chlorite, muscovite, garnet.	Orthoclase, epidote, hornblende, rutile, zircon, muscovite, garnet.	Orthoclase is badly weathered and hornblende shows signs of passing over into epidote. Much secondary quartz here.
Orangeburg (subsoil).	8 to 36	6	9	.....do.....	Orthoclase (residues), microcline.	Orthoclase, epidote, microcline, hornblende, magnetite, zircon, rutile, chlorite, tourmaline, garnet, muscovite.	Hornblende, epidote, magnetite, rutile, zircon, muscovite.	Soil characterized by much-weathered orthoclase. Very similar to the Norfolk series.

An average of five samples of soil of the Porters series, including types of different texture, shows that 52 per cent of the minerals in the very fine sand separates comprises other minerals than quartz. The potash-bearing minerals are decidedly the predominating ones. Biotite and muscovite mica have been found among the predominating minerals in all five samples, having an average of 20 per cent of all the minerals except quartz. Orthoclase is very abundant in the soils of this province; it, too, has been found among the abundant minerals in all five samples. Microcline is often encountered, especially among the sand particles; however, it is not found as abundantly as orthoclase. A study of the optical properties of biotite and orthoclase often shows them to be undergoing well-marked chemical alteration, the former being metamorphosed to chlorite and epidote and the latter wearing down, leaving a somewhat skeleton-shaped residue. Plagioclase[1] feldspars are encountered often in the soils of this locality; in many instances they are found as well-preserved fragments, which show clean faces and sharp edges, as though little decomposition had taken place.

Another point that may be worthy of note is the accumulation of micas in the silt separates. Not only is this true for the soils of the Appalachian Mountains, but it is most frequently the case with other soils of the United States. If these minerals are found in a soil to any appreciable extent, they usually occur in the largest quantities among the finer particles. This is readily accounted for from their cleavage and other physical properties, which cause them to be quite susceptible to the forces of weathering. As these minerals are carriers of the element potassium, practical significance may be attached to this fact. As they occur among the finer particles, more surface is exposed to the forces which make the soil solution, thereby causing more of this element to be of service to plant life than when found among particles of coarser texture.

Pyroxene and serpentine are found in more abundance in the Mountain soils than is usually the case with those of the Piedmont and Coastal Plain provinces.

Apatite, the mineral carrying the element phosphorus, is somewhat more common in these soils. It is found both as prismatic apatite and as tiny needles inclosed in other minerals. Fry [2] has called attention to the persistence of included apatite in soils, which may have some bearing on the availability of this element when so found.

The mineral epidote is often found among the predominating minerals of the soils in all parts of the State. Its persistence is readily explained, as it is a product of the metamorphism of the lesser resistant minerals, biotite and hornblende.

[1] The writer has not attempted to differentiate between the members of the plagioclase group.

[2] Fry, W. H. The condition of phosphoric acid insoluble in hydrochloric acid. *In* Jour. Indus. and Engin. Chem., v. 5, no. 8, p. 664–665. 1913.

Tourmaline, sillimanite, rutile, and zircon persist in many soil series; in fact, in very few in this State are they entirely absent. They are extremely resistant in character, which is undoubtedly the cause of their persistence.

The soils of the Cecil series are by far the most predominating of the Piedmont Plateau. Though formed from the same general character of rocks, they differ decidedly in mineral complexity. The quantity of minerals other than quartz in the Porters series is nearly double that of the Cecil series. However, minerals of nearly the same kind are encountered in both. As a general rule, greater decomposition has taken place among the minerals of the Piedmont soils; especially is this true of the silt particles. In many of the clay types of the Cecil soils biotite mica is found in only minute quantities, which would tend to show that it is passing out of existence in these older soils. Plagioclase feldspars and apatite are found only in very minute quantities in the soils of this series. Even the quartz particles appear to have undergone much greater wearing than in the mountains.

This is in accord with the work of Coffey [1] in showing the effect of topography upon the composition of soils. In the mountains the forces of erosion have not allowed the soil mantle to become as well defined as it is in the Piedmont Plateau; consequently, there is greater preponderance of the minerals found in the parent rocks when the superficial covering is removed. This fact is better illustrated in the accompanying reproductions of photomicrographs of representative soils of the two provinces (Pl. LII). Quartz and some of the other minerals are eliminated in these cuts, but the relative number of minerals other than quartz in the two samples is easily discernible.

The Iredell soils are formed from the basic eruptives, mica diorite, gabbro-diorite, and meta-gabbro. Quartz is a subordinate mineral, for in the sand portions of five samples whose averages were taken 80 per cent of other minerals than quartz is found. Among the silt particles quartz amounts to only about 5 per cent of the total minerals. Epidote, hornblende, and augite compose the greater part of the particles of coarser texture, while biotite and pyroxene are found more abundantly in the silt. Very little decomposition had taken place among any of the minerals found in this series; even the plagioclase feldspars, which occur in rather large quantities, do not show signs of serious chemical decomposition. An interesting point is the scarcity of the potash feldspars, orthoclase and microcline. Apatite is found in much larger proportions than in any other soil series in North Carolina, which is in accord with the total chemical analysis. As an average of five samples of the Iredell loam, the phosphoric-acid content is found to be 6,251 pounds per acre

[1] Coffey, G. N. A study of the soils of the United States. U. S. Dept. Agr. Bur. Soils Bul. 85, 114 p., map. 1912.

for the first 6⅔ inches, which is considerably higher than the average for the soils of the State. Field experiments which have been conducted on this series for the past five years indicate that phosphorus is in no way the limiting element in crop production.

The Granville soils, which are found in limited areas in the Piedmont Plateau, are formed from sandstone and shale. These soils are unusually high carriers of potassium, which is supplied mainly as microcline and orthoclase. While some biotite and muscovite are encountered, very little of the potassium must come from this source. It would be interesting to have field data on the requirements of the soils of this series for potassium, for comparison with those of the Mountain province, in which mica predominates.

The Georgeville soils represent those formed from Carolina slate, and the minerals other than quartz are mainly the potash feldspars and those of a highly refractory character. Many of the particles carry an infiltration of iron oxid, which makes identification quite difficult. Much of the orthoclase and biotite is badly altered, while other particles of these minerals are found in an unusually fresh condition, which indicates that an admixture of the material which enters into the formation of this soil has taken place.

The soils of the Atlantic Coastal Plain are characterized by their low content of other minerals than quartz. The Norfolk and Portsmouth series are by far the prevailing soils of this province, and, with few exceptions, no particular mineral other than quartz predominates. It might be said in passing that a few instances occur in which the other minerals than quartz will run higher, but this is unusual.

The average among the sand particles for the Norfolk series will not exceed 5 per cent of minerals other than quartz, of which none predominate. Among the particles the size of silt will be found orthoclase residues, microcline predominating. The less abundant minerals are composed mainly of a heterogeneous mixture of the more refractory minerals found in the provinces of higher topography. A point of interest is the scarcity of the micas in the series; they are encountered often, but the quantity found is usually so small that they can be of little value in maintaining the potash content of the soil solution. Apatite and the plagioclase feldspars are rarely found, as they have passed out of existence during the formation of this soil.

The Portsmouth soils are quite similar to the Norfolk, the only distinctive difference being in the amount of organic matter found in the former. On account of their location, which is usually in submerged or recently drained areas, an accumulation of vegetable matter is encountered. The average content of minerals other than quartz in this series is even lower than that of the Norfolk, being 3 per cent. The persistence of sponge spicules or Rhizopoda casts in this series is rather

interesting. These ham-shaped, isotropic particles are the remains of some form of life that flourished here during the submergence of this land.

In the Orangeburg series occurs a higher content of minerals other than quartz than is found in either the Norfolk or the Portsmouth series, but still the amount is small. The soils of the Orangeburg series resemble the Norfolk in many respects, and the same general minerals are encountered.

The low content of other minerals than quartz in the soils of the Atlantic Coastal Plain is in close agreement with the total chemical analyses of the three plant-food constituents—phosphoric acid, potash, and lime. Many chemical analyses of the soils of this province show the above-named elements of plant growth to be exceedingly low. Not only do there appear to be close relationships existing between the total chemical analyses and their mineralogical complexity here, but in the soils of the entire State. This would suggest that since the petrographic methods have reached so high a state of development they may be used with a fair degree of accuracy for estimating the amounts of the mineral plant-food constituents carried by a soil. On account of the ease of manipulation and the time saved in their use, they lend themselves readily for such purpose; especially is this true in scanning soils for the farmer. The information gained is usually not commensurate with the time and expense involved in making "bulk analyses" of soils for farmers. As a rule, it is not necessary that he know the exact number of pounds of plant food contained in his soil; an approximation will usually suffice. A very close estimate as to the quantity of the elements present may be easily secured with the microscopic methods; even more, the way these elements are held is revealed. If more data were at hand showing the availability of the various mineral elements of plant growth furnished by the different soil-forming minerals, more definite information could be obtained as to the fertilizer requirements of the land with the microscope than by "bulk analyses."

In a former publication [1] the writer submitted data from which there appeared to be some relationships existing with certain crops between the mineralogical and chemical composition of the soils of this State and their requirements for the inorganic elements found in the usual fertilizer mixture—namely, phosphoric acid, potash, and lime. Additional evidence will be submitted along this line, using the cotton plant as the indicator for measuring the relative densities of the soil solution.

In Table II will be found the average results of seven years' fertilizer treatments with cotton at the Iredell Substation, located upon typical Cecil clay loam.

[1] Plummer, J. K. Relation of the mineralogical and chemical composition to the fertilizer requirements of North Carolina soils. N. C. Agr. Exp. Sta. Tech. Bul. 9, 29 p. 1914.

TABLE II.—*Average yield of cotton on fields A, B, and C, with seven years' fertilization at the Iredell Substation*

Treatment.	Average yield of seed cotton per acre.			Average increase per acre due to fertilizer.
	Field A (1903, 1904, 1906, and 1909).	Field B (1905 and 1907).	Field C (1908).	
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
Nitrogen	210. 6	377. 5	505. 0	—11. 7
Phosphoric acid	655. 6	897. 5	860. 0	441. 8
Potash	301. 3	537. 5	435. 0	85. 4
Nitrogen and phosphoric acid	897. 5	727. 5	620. 0	520. 1
Nitrogen and potash	348. 8	406. 3	400. 0	96. 5
Phosphoric acid and potash	855. 0	959. 8	725. 0	608. 0
Nitrogen, phosphoric acid, and potash	923. 8	1, 002. 3	1, 070. 0	717. 7
Lime	97. 5	160. 0	430. 0	27. 0
Lime, nitrogen, phosphoric acid, and potash	728. 8	637. 5	945. 0	573. 5

A glance at Table II will show that phosphoric acid is the limiting or controlling element of plant growth for this soil. An average increase for the seven years' treatment of 441.8 pounds is obtained with phosphoric acid alone, while there was an increase of only 85.4 pounds with potash and no increase at all with nitrogen. Nitrogen added to phosphoric acid produced but a slight increase over the latter constituent alone, while potash added to phosphoric acid produced a somewhat better yield.

Table III shows an 8-year average with cotton at the Experiment Station Farm at Raleigh with typical Cecil sandy loam.

TABLE III.—*Average yield of cotton on fertilized fields A and B at the North Carolina Experiment Station Farm, Raleigh*

Treatment.	Average yield of seed cotton per acre.		Average increase per acre due to fertilizer.
	Field A (1902, 1903, 1904, 1906, and 1908).	Field B (1905, 1907, and 1909).	
	Pounds.	*Pounds.*	*Pounds.*
Nitrogen and phosphoric acid	1, 154. 5	768. 2	415. 1
Nitrogen and potash	994. 6	437. 7	169. 9
Phosphoric acid and potash	1, 126. 0	895. 3	464. 4
Nitrogen, phosphoric acid, and potash	1, 130. 8	925. 7	524. 6
Lime	619. 5	320. 1	31. 9
Nitrogen, phosphoric acid, potash, and lime	1, 007. 2	975. 3	572. 7

The 8-year average with cotton given in Table III again shows that phosphoric acid is the controlling element in these fertilizer tests. When potash and nitrogen are used in quantities, as in this experiment, only slight increases in yield are produced. The former constituent gave a slightly greater average than did the latter. The average "bulk analyses" of many samples of soil from these two fields, as well as from Norfolk fine sandy loam, will be found in Table IV.

TABLE IV.—*Average quantity of the total plant-food constituents per acre in various types of soil*

SURFACE SOIL TO DEPTH OF 6⅔ INCHES (2,000,000 POUNDS)

Soil type.	Nitrogen (N).	Phosphorus pentoxid (P_2O_5).	Potassium oxid (K_2O).	Calcium oxid (CaO).
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
Cecil clay loam	1,141	1,155	7,213	4,656
Cecil sandy loam	769	503	2,994	5,542
Norfolk fine sandy loam	853	953	3,087	3,220

SUBSOIL TO DEPTH OF 28 INCHES (8,000,000 POUNDS)

Cecil clay loam	2,378	9,169	25,090	19,933
Cecil sandy loam	1,993	4,007	19,073	26,512
Norfolk fine sandy loam	1,360	1,573	11,453	8,880

A comparison of the yields of cotton on the two fields shows marked similarity in fertilizer requirements though the fields are over 100 miles apart. These soils belong to the same series, though of decidedly different texture, one being a rather heavy clay, the other a medium sandy loam. Unquestionably there are numerous other factors than the amount of plant food carried by the two soils which enter into their productiveness; nevertheless, some relationships exist between this question and their requirements for these fertilizer elements. As shown in Table IV, the phosphoric-acid content of both soils is low; until this element has been added in sufficient quantities there can be no increase yields. Although the nitrogen supply in the two soils is found in about the same proportion as the phosphoric acid, it is evidently changed into a more available form faster than the latter element.

The potash content of the Cecil clay loam is about double that of the Cecil sandy loam, both soils showing that potash is in no way the limiting element. Indeed, it is doubtful whether this element can be applied to the former at a profit. A glance at Table I, which gives the mineral composition of the Cecil series, shows that in the fine sand and silt separates the potash minerals predominate and that biotite mica is found among the abundant minerals in all five samples.

Lime has not given material gains with cotton in either test, owing undoubtedly to the physical condition of this land and the large amount of lime carried by the two soils. As a general rule, the minerals which carry lime in the Piedmont soils are more susceptible to chemical and physical decomposition than those found among the fields of the Atlantic Coastal Plain.

Table V gives the average yield of cotton on Norfolk fine sandy loam at the Edgecombe Substation with seven years' fertilization.

TABLE V.—*Average yield of cotton on fields A and B with seven years' fertilization at the Edgecombe Substation*

Treatment.	Average yield of seed cotton per acre.		Average increase per acre due to fertilizer.
	Field A (1903, 1904, 1906, and 1908.)	Field B (1905, 1907, and 1909.)	
	Pounds.	*Pounds.*	*Pounds.*
Control	1,030	429	.....
Nitrogen and potash	1,215	1,059	376
Phosphoric acid and potash	1,076	873	217
Nitrogen, phosphoric acid, and potash	1,193	1,022	348
Nitrogen and phosphoric acid	1,108	717	167
Lime	1,061	510	62
Lime, nitrogen, phosphoric acid, and potash	1,441	1,024	499

Table V gives the results of fertilizer tests which are in marked contrast to those obtained from the Cecil series of the Piedmont Plateau. Fertilizer mixtures carrying potash give the most marked yields; in fact, nitrogen and potash give greater returns than the three fertilizer constituents.

Lime in connection with the three fertilizer elements has produced decided gains. The physical condition of this soil is surely as good as that of the Cecil sandy loam at Raleigh, and the amount carried by the soil is quite sufficient to furnish this constituent as a plant food for a number of years to come. The petrographic examination of the Norfolk soils gives epidote as the only lime-bearing mineral of any consequence. It would seem therefore that lime carried in this form is of doubtful value in performing its functions in the soil.

The amount of potash here is even greater than that found in the sandy loam at Raleigh, yet potash seems to be the limiting element on this field. Weathered orthoclase and microcline furnish practically all the potash supply of this soil, while biotite and muscovite micas are much more abundant in the Cecil series.

Another interesting point brought out in these experiments is in regard to the phosphoric-acid content of the three fields. In the Edge-

combe field the content of phosphoric acid is somewhat less than that of the Cecil clay loam at the Iredell farm, yet in the latter soil phosphorus is the limiting element; but this is not the case in the former, owing doubtless to the way this constituent is held in the two soils. The supply of phosphorus must be stored in the organic form. There is practically no apatite in this Norfolk soil, while it is readily encountered in the residual soils of the Piedmont Plateau, occurring both free and included in quartz and other minerals.

CONCLUSIONS

The results of this and other work on the subject indicate that the following conclusions can be drawn, some of which are undoubtedly applicable to other than North Carolina conditions.

Wide variations in mineralogical composition are found between the soils of the Appalachian Mountains, Piedmont Plateau, and Atlantic Coastal Plain. There is unquestionably a greater supply of minerals which carry the inorganic plant-food constituents in the Mountain soils than are found in either the Piedmont Plateau or the Coastal Plain. Though many of the former soils are derived from the same rocks as those of the Piedmont province, the forces of erosion among those of the mountains cause them to contain minerals more nearly the same as the parent rocks than are found elsewhere.

Definite information is required on the behavior of the various soil-forming minerals to the forces of weathering before positive conclusions can be drawn on the availability of the plant food carried by the different minerals.

The field results with the cotton plant indicate that there are some relationships existing between the mineral component of the soil and the requirements of this plant for the three inorganic fertilizer constituents, phosphoric acid, potash, and lime.

PLATE LII

Fig. 1.—Photomicrograph of Porters soil of the Appalachian, No. 5 sand.
Fig. 2.—Photomicrograph of Cecil soil of the Piedmont Plateau, No. 5 sand.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., JANUARY 3, 1916 NO. 14

HOURLY TRANSPIRATION RATE ON CLEAR DAYS AS DETERMINED BY CYCLIC ENVIRONMENTAL FACTORS

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INTRODUCTION

The great differences exhibited by various plants in water requirement—i. e., in the water transpired in the production of a unit of dry matter—are of profound economic importance in the agricultural development of regions of limited rainfall, and an understanding of what gives rise to the greater efficiency which some plants possess in the use of water is highly desirable in the selection and breeding of plant strains adapted to dry-land agriculture. This problem has led the writers to undertake a series of transpiration measurements with a view to determining, so far as possible, the relative influence of various environmental factors on the transpiration of different plants. To this end simultaneous automatic records have been obtained of the solar-radiation intensity, the depression of the wet-bulb thermometer, the air temperature, the wind velocity, and the evaporation from a free-water surface. The present paper deals with the transpiration response of plants to these factors on clear days.

DESCRIPTION OF APPARATUS AND METHODS

MEASUREMENT OF TRANSPIRATION

The transpiration measurements described in this paper were carried out at Akron, Colo., in 1912, 1913, and 1914 (Pl. LIII). Transpiration was determined by weighing, four large automatic platform scales of a type already described (Briggs and Shantz, 1915)[2] being used in these measurements. The plants employed were those used in the water-

[1] The writers desire to express their indebtedness to the following men for efficient and painstaking assistance in connection with data presented in this paper: Messrs. F. A. Cajori, R. D. Rands, A. MacG. Peter, J. D. Hird, R. L. Piemeisel, P. N. Peter, H. W. Markward, G. Crawford, and H. Martin.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 648–649.

requirement investigations, and were grown in the sealed pots already described (Briggs and Shantz, 1913, p. 9), which practically eliminate the direct loss of water from the soil. The pots contained about 115 kgm. of soil and were sufficiently large to enable the plants to make a normal growth, a factor of importance in transpiration measurements (Pl. LV, figs. 1–2). A part of the transpiration measurements were made within the screened inclosure (Pl. LIV, fig. 1) used in the water-requirement experiments to protect the plants from hail and wind storms. Other measurements were made outside the inclosure where the plants were freely exposed, with no protection whatever (Pl. LIV, fig. 2).

MEASUREMENT OF PHYSICAL FACTORS

SOLAR RADIATION.—The solar-radiation measurements were made automatically with a mechanical differential-telethermograph already described by one of the writers (Briggs, 1913). The instrument has two independent cylindrical bulbs and records only the difference in temperature of the two bulbs. When used for measuring radiation intensity, one bulb is blackened and surrounded by a spherical glass envelope (Pl. LIII). This is so exposed to the sun that the longer diameter of the bulb is normal to the sun's rays at midday. This bulb rises in temperature until the rate at which energy is lost is equal to the rate at which it is received. The other bulb follows the temperature of the air within the instrument shelter, through which the wind blows freely. The instrument was calibrated by comparison with an Abbot silver-disk pyrheliometer (Abbot, 1911). Such comparison shows that the difference in temperature, as measured by the telethermograph, is very nearly proportional to the intensity of the solar radiation falling on a blackened surface normal to the ray, as measured by the pyrheliometer. In other words, the scale is linear and the loss of energy conforms to Newton's law of cooling. While the telethermograph includes the sky radiation as well, the apparatus can be calibrated in terms of the solar radiation on bright days, since on clear days the ratio of sun to sky radiation appears to be fairly constant and the latter at the elevation of Akron (4,200 feet) is small compared with the direct radiation from the sun. A comparison of the telethermograph with the pyrheliometer, when the former is used for measuring radiation, is given in figure 1.

The radiation data given in this paper are expressed in terms of differential temperatures and the mean values are converted to calories per square centimeter per minute on a surface normal to the sun's rays.[1] The radiation is integrated for hourly periods so that zero radiation is not recorded until the hour following the hour interval during which the sun set, or preceding the hour interval during which the sun rose.

[1] The magnification of the differential sunshine instruments was not the same in 1912 and 1914. To convert to calories per square centimeter per minute multiply the differential temperatures in the 1912 observations by 0.0335; and in the 1914 observations by 0.028. In the 1914 observations the instruments were so adjusted as to give differential temperatures in degrees Fahrenheit.

WET-BULB DEPRESSION.—The measurement of the depression of the wet bulb was automatically carried on by means of a second differential telethermograph. One bulb was surrounded by muslin which was kept continuously saturated with water by means of a slowly-dripping Mariotte apparatus. In these measurements both bulbs were inside the instrument shelter and protected from solar radiation. The apparatus thus measured the depression of the wet bulb corresponding to the ventilation afforded by the wind through the shelter, which was similar to that to which the plants were subjected.

EVAPORATION.—In measuring the evaporation a shallow copper tank 91.3 cm. (3 feet) in diameter and 2.5 cm. deep was used, being mounted on the platform of an automatic scale of the type used in the transpiration measurements. The tank was clamped to a heavy, flat, wooden base,

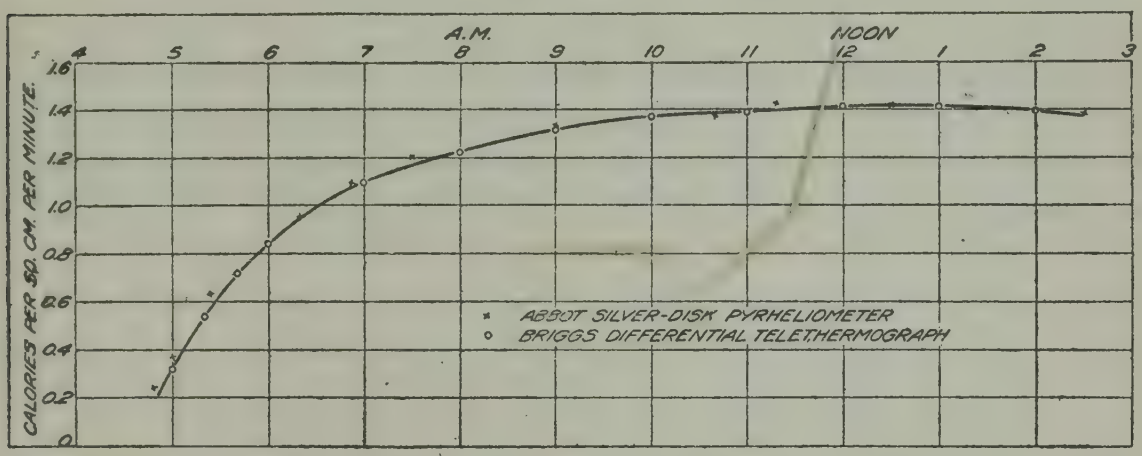

FIG. 1.—Curve showing the comparison of the readings of the differential telethermograph with those of Abbot's silver-disk pyrheliometer.

which was supported on leveling legs about 3 feet above the scale platform (Pl. LV, fig. 3). The inside of the tank was blackened with a mixture of lampblack in "bronzing liquid." The depth of the water in the tank was maintained at approximately 1 cm. by means of a Mariotte apparatus supported from the scale platform and located on the north side of the tank, so that its shadow did not fall on the tank.

AIR TEMPERATURE.—The air temperature was measured by a thermograph calibrated with mercurial thermometers and exposed in a standard shelter of the Weather Bureau pattern.

WIND VELOCITY.—The wind velocity was measured automatically by an anemometer of the Weather Bureau pattern, located 3 feet above the ground. In the 1914 measurements these measurements were supplemented by a special instrument recording each one-twentieth of a mile.

TRANSPIRATION RATE ON CLEAR DAYS IN RELATION TO PHYSICAL FACTORS

The transpiration graph for a single pot of plants for a single day usually shows slight irregularities. In order, therefore, to determine whether such departures are normal or accidental, it is necessary to combine

the transpiration graphs for a number of clear days sufficient to eliminate or minimize the accidental features. In the same manner a composite graph for the corresponding days can be prepared for each of the cyclic environmental factors—radiation, temperature, and wet-bulb depression. The evaporation data have also been combined in the same way. This procedure is not adapted to factors which are essentially noncyclic in character. Wind velocity, for example, is essentially cyclic in some regions and noncyclic in others. While the wind at Akron gives evidence of a daily periodicity, the cyclic character is not sufficiently developed to give the composite graph much weight. The discussion is not, however, limited to the composite values, the hourly values of the transpiration and of each environmental factor being given in the tables for each day considered.

WHEAT

The data obtained for the transpiration of wheat (*Triticum* spp.) on clear days in 1912 are given in Table I, and the environmental data for the corresponding period, including solar radiation, air temperature, and wind velocity in Tables II, III, and IV, respectively.

TABLE I.—*Transpiration rate (in grams per hour) of wheat, at Akron, Colo., during June and July, 1912*

Variety.	Balance No.	Date.	Hour ending— A.M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8	9	10	11	12
Turkey	B	June 25	6	6	6	8	10	20	80	140	180	220	240	240	240	240	270	240	220	180	94	30	12	12	10	10
Do	B	26	16	12	12	14	16	60	110	140	140	170	170	200	220	210	250	240	200	180	74	14	12	14	12	12
Do	B	27	20	14	8	6	16	20	140	200	240	240	250	260	260	260	270	250	200	190	46	26	26	14	10	10
Do	B	28	10	8	8	8	8	20	110	140	210	270	270	260	280	320	310	290	240	160	70	20	20	30	16	12
Do	B	July 5	6	6	12	12	12	20	60	130	150	170	180	180	180	180	170	170	140	120	60	22	20	12	12	12
Do	B	7	14	4	4	4	4	40	60	140	160	180	170	180	200	220	240	220	190	140	70	20	20	20	20	20
Do	B	11	20	16	12	14	2	50	60	160	220	220	240	280	280	300	300	260	240	200	100	40	12	20	20	20
Average for Turkey			13.1	9.4	8.9	9.4	9.7	32.8	88.5	150	186	210	217	229	237	247	258	239	204	167	73.4	24.5	17.4	17.4	14.3	13.7
Kubanka	A	June 25	4	4	4	4	4	20	40	60	90	100	120	120	120	140	140	130	110	90	40	6	6	4	4	4
Do	A	26	4	4	4	4	4	40	56	86	84	100	110	110	120	120	130	120	110	94	46	4	4	4	4	4
Do	A	27	4	4	4	4	4	40	80	90	110	100	110	120	140	130	140	120	110	80	20	12	2	2	2	2
Do	A	28	4	4	4	4	4	20	50	90	100	130	120	140	150	130	160	160	160	100	50	10	10	4	4	4
Do	A	29	4	4	4	4	4	70	90	120	140	140	140	160	170	170	150	140	130	80	30	14	4	4	4	4
Do	A	July 5	4	4	4	4	10	32	50	80	120	130	130	140	150	160	160	150	140	100	50	14	4	4	4	4
Do	A	7	3	3	3	3	4	24	60	120	170	160	180	180	200	220	250	200	180	140	50	16	12	6	6	6
Do	A	8	8	8	8	6	6	30	100	140	170	190	220	220	250	250	260	260	250	200	110	16	10	10	10	10
Average for Kubanka			4.4	4.4	4.4	4.1	5	34.5	65.7	98.2	123	131	141	149	162.5	165	174	160	149	110.5	49.5	11.5	6.5	4.8	4.8	4.8
Kharkov	C	June 20	4	4	4	4	4	26	60	140	180	240	240	250	270	290	260	260	220	170	80	16	12	12	12	10
Do	C	27	18	14	12	12	26	70	180	320	280	290	300	280	280	300	280	310	290	200	50	30	6	14	10	8
Do	C	July 7	16	12	8	6	8	12	80	160	180	230	200	240	230	280	300	280	230	180	114	30	30	30	30	30
Do	D	June 28	10	10	10	10	14	40	150	220	270	320	330	350	420	420	420	450	390	240	120	30	20	28	26	18
Do	D	July 2	4	4	4	4	10	30	120	160	200	230	240	280	280	320	360	340	250	190	70	24	10	10	12	10
Do	D	5	5	5	5	5	12	36	60	160	190	230	220	210	210	200	180	170	140	116	70	26	14	14	14	14
Average for Kharkov			9.5	8.2	7.2	6.8	12.3	35.7	108.3	193.4	217	257	255	268	282	302	300	302	253	183	84	26	15.3	18	17.3	15
Average, all varieties			8.8	7.1	6.7	6.7	8.7	34.3	85.5	142.7	170.7	193.3	199	209.5	221.4	231.4	238.1	226.7	192.3	150	67.3	20	12.7	12.8	11.5	10.6
Percentage of maximum			4	3	3	3	4	14	36	60	71	81	84	88	93	97	100	95	81	63	28	8	5	5	5	4

TABLE II.—*Hourly solar radiation intensity (observed differential temperatures) during wheat transpiration period, at Akron, Colo., during June and July, 1912*

Date.	Weight.	Hour ending—														
		A. M.							Noon.	P. M.						
		5	6	7	8	9	10	11	12	1	2	3	4	5	6	7
June 20	1	5	7	13	16	19	21	21	21	21.5	20	17	14	10	7	0
25	2	5	10	15	19	21	23	24	25	24	24	23	20	11	10	2
26	2	2	10	16	20	21	23.5	23.5	25	25.5	25.5	24.5	21	18	6	0
27	3	1	10	15.5	18.5	20	21.5	24	25	24	23	23.5	20	15	9	0
28	3	0	8	16	18	21	24	25	25.5	26	26	24	19.5	15	6	2
29	1	3.5	10	15.5	18	20.5	21.5	22	22	22	21.5	20	14	6	1	0
July 2	1	4	10	16	18	21.5	23	23.5	23.5	23	22.5	21	14	7	9	0
5	3	1	5	15	19	21	22	23.5	23.5	23	22.5	20	17	13	9	1
7	3	0	9	15	18	22	24	24	23.5	24	22.5	22.5	15	13	6	0
8	1	0	9	17	18.5	19.5	21	22	22.5	23	21	19.5	18	13.5	8	3.5
11	1	0	10	15	18	20	21	21.5	22	21	10	11	4	5	6	0
Average		1.6	8.7	15.4	18.4	20.8	22.6	23.6	24.0	23.8	22.7	21.6	17.2	12.7	7.3	.8
Calories per square centimeter per minute		.05	.29	.52	.62	.70	.75	.79	.80	.80	.76	.72	.58	.43	.24	.03
Percentage of maximum		7	36	64	77	87	94	98	100	99	95	90	72	53	30	3

TABLE III.—*Hourly temperatures (in degrees Fahrenheit) during wheat transpiration period, at Akron, Colo., during June and July, 1912*

Date.	Weight.	Hour ending—																						
		A. M.											Noon.	P. M.										
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 20	1	48.5	48	47.5	48	49	52.5	59	63	68	71	73.5	75	76.5	77	77	77.5	77	75	69	66	61	58	56	55
25	2	59	58	56	55.5	55	57	63	68	74	78	81	83	84	85	85.5	85	84	83	79	70	64	62	61	60.5
26	2	59	58	56.5	56	56	58	65	69	73	78	83	84	85.5	86	86.5	86	85	83.5	77	71	64	62	61	60.5
27	3	61.5	63	62	61	58	59	68	75	79	81.5	83	85	86.5	87.5	88	88	87	85	78	72.5	71	68	65	62
28	3	59	58.5	58	58.5	57.5	58	66	70	76	81	83	85.5	87	88	88.5	88.5	87	84	79	75	70	68.5	69	68
29	1	66	63.5	63	61.5	59	60	64	70	74	78	81	83.5	87	87.5	88.5	88	86	84	79	75	72.5	72.5	71.5	69.5
July 2	1	53.5	52.5	50.5	50	50.5	54	63	67	71	74	77.5	80	82.5	84	84	83.5	80	77.5	73	70.5	68	66	63	62.5
5	3	49	48.5	49.5	49	49	52	55	59	64	68	70	72	74	75	76	76	76.5	75.5	72	67	61	58.5	55	55
7	3	65	63	61	58	55	58	68	72.5	77	80	83	86	89	91	90	88	87	85	79	73	75	77	71	68
8	1	61	60	59	60	60.5	62	69	77	82	86	90.5	92.5	93.5	95.5	96	95.5	94	92	84	77	68	64	64	63.5
11	1	64	63	61.5	61	61	62.5	66	70	77	81	84	86	89	90	91.5	91	87	81.5	76	71.5	67	63.5	61.5	62
Average		58.7	58	57	56.3	55.5	57.2	64.2	69.1	74.0	77.8	80.6	82.7	84.6	85.8	86.1	85.7	84.4	82.5	77.0	71.6	67.8	66.1	63.8	62.6
Average in degrees centigrade		14.8	14.4	13.9	13.5	13.1	14.0	17.9	20.6	23.3	25.4	27.0	28.2	29.2	29.9	30.1	29.8	29.1	28.1	25.0	22.0	19.9	18.9	17.7	17.0
Percentage of maximum range		10	8	5	3	0	6	28	44	60	73	82	89	95	99	100	99	94	88	70	53	40	35	27	23

TABLE IV.—*Wind velocity in miles per hour during wheat transpiration period at Akron, Colo., during June and July, 1912*

Date.	Weight.	Hour ending—																						
		A. M.											Noon.	P. M.										
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 20	1	.....	.....	.....	.....	.....	.....	.....	5	4	3	3.8	3	2.7	2.7	3.1	2.4	1.5	7.6	2	1	1.5	0.8	0.2	1.2
25	2	3.0	3.0	2.0	3.0	3.0	3.0	6	7	6	5.5	5.5	4.5	4.5	4.7	5.0	6.0	5.2	2.7	1.5	1.5	1.2	2	3	1
26	2	3.0	2.5	2.0	4.0	5.0	4	7.2	9.5	5	4.2	2.9	3.0	1.5	1.5	1.3	2.3	3.9	4.1	2.0	1.5	1.5	1	4	4
27	3	4.0	6.0	5.2	4.0	3.5	4.5	5.5	8.6	10.8	13.2	12.8	12.6	10.7	10	9	9.2	5.6	7.5	7.0	4.0	3.0	2.0	3.0	4.0
28	3	1.0	2.5	3.0	3.5	3.0	5.0	.....	4.3	4.5	4.6	3.2	3.0	5.5	7.0	6.2	6.7	6.0	4.0	1.5	1.5	1.0	2.0	.....	.....
29	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
July 2	1	1.0	1.0	.5	.5	.5	.5	4.2	6.0	3.8	5.3	2.8	3.0	4.0	4.3	4.0	4.5	7.0	10.5	1.0	7.0	4.0	5.0	2.0	4.0
5	3	1.0	4.0	2.5	1.5	5.0	5.0	.....	8.0	8.5	8.0	7.7	7.3	7.0	6.0	4.0	3.7	2.5	2.0	1.5	.5	1.0	.5	.5	1.0
7	3	8.5	5.0	5.0	1.5	1.5	2.0	4.3	10.0	9.0	6.5	4.0	3.0	2.7	3.1	6.0	8.0	8.0	7.1	3.0	.5	1.5	3.0	2.0	2.0
8	1	2.0	2.0	5	6.0	6.0	4.0	4.7	4.0	5.0	5.5	7.5	5.2	3.4	5.5	6.3	4.0	6.2	5.5	3.5	1.5	1.0	1.5	2.0	1.5
11	1	5.0	4.5	7.5	6.0	7.0	9.0	9.3	8.3	5.7	3.7	3.0	2.4	2.2	2.6	2.0	2.9	7.5	12.0	4.0	1.0	0.7	0.3	1.5	4.0
Average		3.3	3.7	3.6	3.1	3.6	4.1	5.7	7.5	7.0	6.7	5.9	5.3	5.1	5.3	5.2	5.7	5.3	5.3	2.8	1.8	1.6	1.8	2.1	2.5

The mean values are plotted in figure 2. It should be recalled that all transpiration measurements in 1912 were made in the hail-screen inclosure (Pl. LIV, fig. 1). The radiation measurements were likewise made under this screen, which reduced the radiation about 20 per cent (Briggs and Shantz, 1914, p. 3). It should also be borne in mind that during the year of 1912 the solar radiation outside the inclosure was about 20 per cent lower than normal (Briggs and Shantz, 1914, p. 54).

The mean solar radiation shown in the first curve of figure 2 is relatively symmetrical, as would be expected if clear or only slightly cloudy days

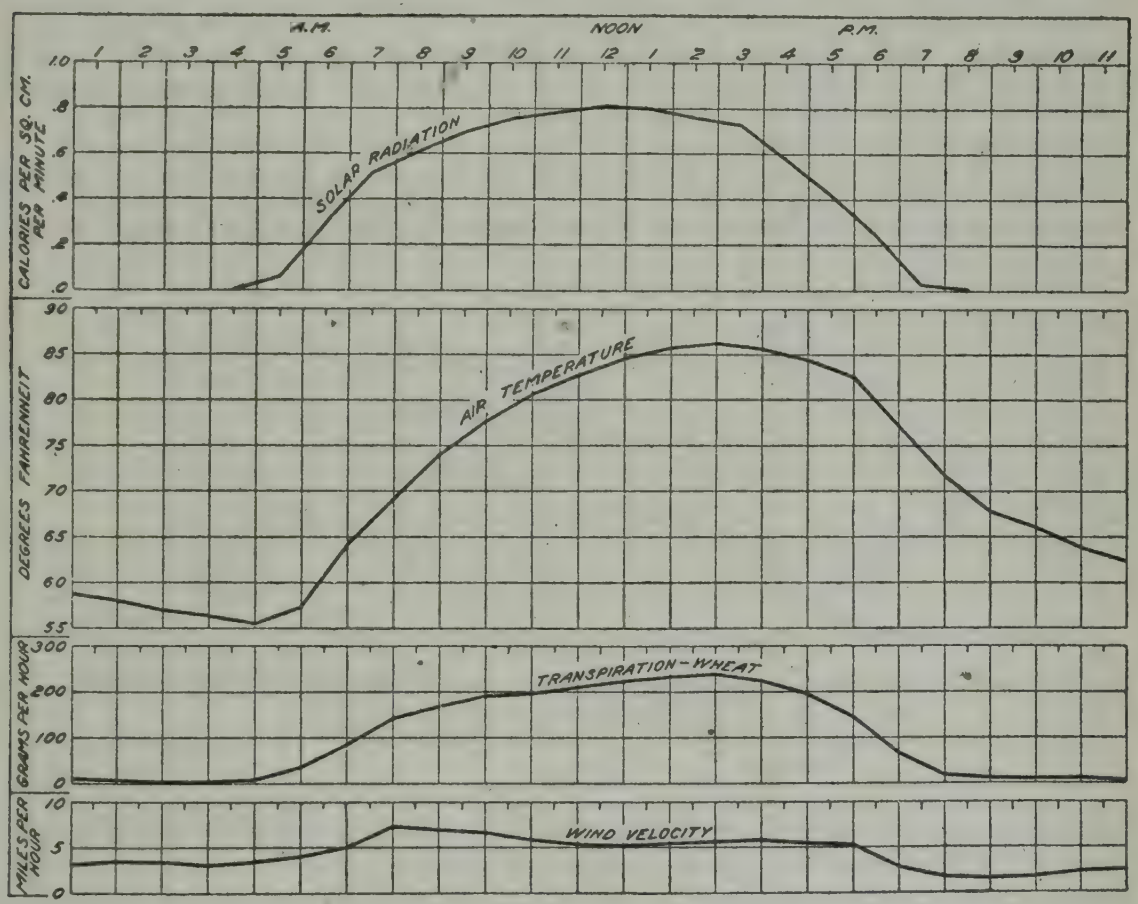

FIG. 2.—Composite transpiration graph of wheat and environmental graphs for corresponding period.

are chosen. The maximum radiation is reached at 12 o'clock, noon, and amounts at that time to only 0.80 calories per square centimeter per minute. The gradient is steep during the early morning and late afternoon, but there is little change in the radiation intensity during the midday hours.

The second graph in figure 2 gives the hourly air temperature in degrees Fahrenheit. The temperature reaches its minimum, 55° F., between 4 and 5 a. m., and its maximum, 86° F., between 2 and 3 in the afternoon. The average temperature from noon to midnight is much higher than from midnight to noon.

The transpiration is recorded in grams per hour. It will be seen from the graph in figure 3 that the transpiration during the night is almost negligible. A marked increase is recorded at 6 o'clock in the morning.

The maximum of 238 gm. per hour is reached about 2.30 p. m., after which the transpiration falls rapidly and acquires the average night rate soon after sunset. There is an indication from the flattening of the curve after 8 o'clock a. m. that from this point on to the maximum the plant modifies its transpiration coefficient.[1] This may be in part due to the closing of the stomata during this period and in part to the lowering of the vapor pressure of the sap of the mesophyll cells resulting from an increase in concentration.

At the bottom of figure 2 is shown the mean wind velocity for each hour of the day. It will be seen that the maximum rate is reached about 7.30 a. m. There is a gradual falling off until about noon, after which the wind velocity remains constant until 5.30 p. m. During the night the rate is somewhat lower.

The transpiration graph of wheat in figure 2 is a composite based upon transpiration measurements of Kharkov and Turkey winter wheats, both

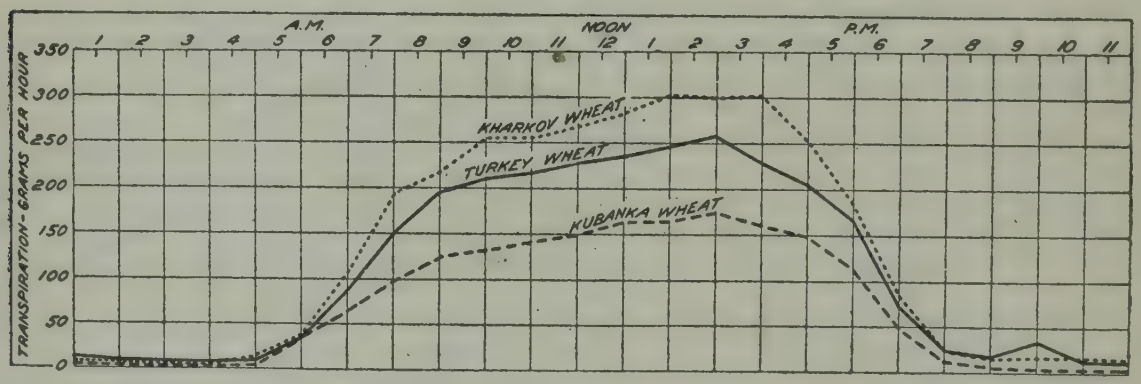

FIG. 3.—Composite transpiration graphs for the three varieties of wheat from which the composite graph of figure 2 was obtained.

being varieties of *Triticum aestivum*, and of one hard spring wheat, Kubanka, a variety of *Triticum durum*.[2] The transpiration graphs for each variety, based upon the data given in Table I, are presented in figure 3. It will be noted that the graphs have essentially the same form and that each graph after 9 a. m. shows a falling off in the transpiration rate below that indicated by the slope during the early morning hours.

OATS[3]

The data covering the transpiration measurements of oats (*Avena sativa*) on clear days are presented in Table V and the environmental measurements for the corresponding period in Tables VI to IX. The

[1] If a plant in its transpiration response to its environment acted as a free physical system, it would be possible to express the transpiration rate in the form of an equation involving the intensity of each of the individual environmental factors. If the relative part played by each factor in determining transpiration were known, then simply by determining the transpiration rate corresponding to some given environment, the transpiration rate for any other environment could be computed. The ratio of the transpiration rate to the environmental intensity would then be defined as the *transpiration coefficient* of the particular plant under observation.

[2] Kharkov, C. I. (Cereal Investigations) No. 1583; Turkey, C. I. No. 1571; and Kubanka, C. I. No. 1440.

[3] Swedish Select, C. I. No. 134.

mean hourly values for each environmental factor and for the transpiration are plotted in figure 4. The graphs for the physical factors represent in each case the mean hourly values for eight clear days. The

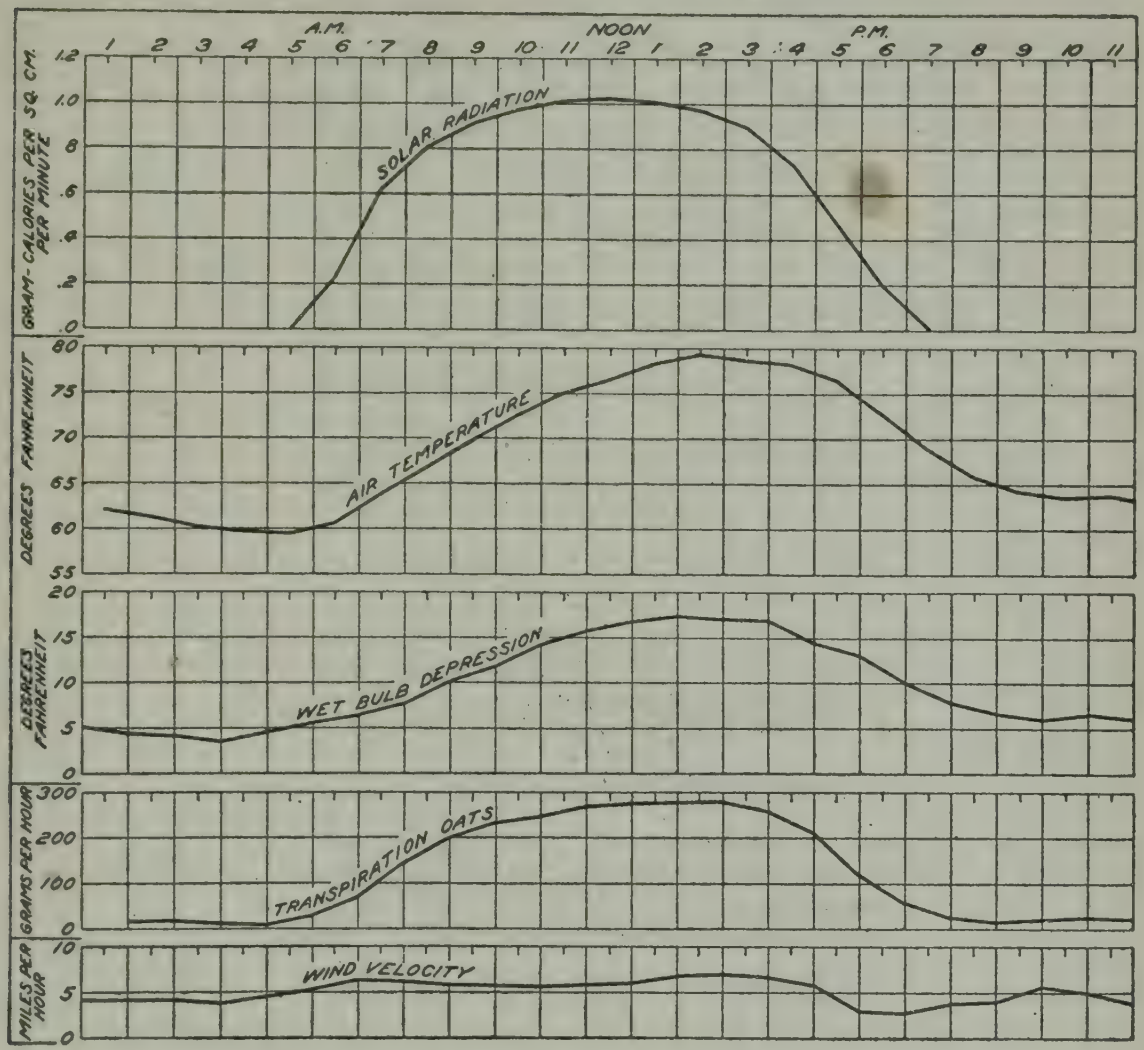

FIG. 4.—Composite transpiration graph for oats, with environmental graphs for corresponding period.

transpiration measurements were made in duplicate, using two pots of oats of the same variety, each pot being mounted independently on an automatic balance. The hourly transpiration values plotted in figure 4, therefore, represent the mean of 16 determinations.

TABLE V.—*Transpiration rate (in grams per hour) of oats at Akron, Colo., from August 4 to 18, 1912*

Date.	Balance No.	Hour ending— A.M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8	9	10	11	12
Aug. 4	C	33.2	4	8	8	12	20	50	134	276	314	374	406	400	380	300	220	144	62	28	14	10	24	28	58
	D	36	3	6	6	6	12	36	114	272	316	362	394	464	324	280	214	150	62	32	12	10	20	20	56
5	C	6	4.2	4.2	4.4	4.4	3	92	130	200	202	200	250	296	330	344	330	287	190	52	18	10	8	46	8
	D	6	14.6	6	6	8.4	22	114	146	194	216	220	266	325	340	348	334	280	186	54	20	24	10	45.4	6
8	C	8	.....	.....	.....	.....	.....	66	144	168	202	190	210	186	180	202	198	164	126	42	16	14	18	10	12
	D	6	.....	.....	.....	.....	.....	.....	132	146	242	192	180	164	166	224	248	170	134	48	18	16	14	10	8
9	C	.....	8	6	6	6	.....	46	136	186	218	240	236	260	260	264	236	230	180	62	12	6	6	.....	.....
	D	.....	6	6	6	8	10	54	144	200	224	236	280	294	284	300	264	216	174	72	14	8	6	6	4
11	C	46	22	48	4	4	6	6	62	244	250	270	296	300	340	340	340	300	216	96	32	22	20	24	32
	D	44	34	34	34	36	48	110	170	280	280	304	316	276	304	344	356	316	236	96	38	22	20	22	30
12	C	10	36	34	26	18	48	110	198	220	250	244	274	264	296	282	276	230	196	72	18	44	18	18	12
	D	14	40	34	26	26	50	122	238	264	276	320	306	294	330	310	296	252	200	74	24	38	18	14	26
13	C	.....	22	10	12	8	2	44	120	164	232	248	250	236	276	258	254	156	54	14	24	.....	.....	.....	.....
	D	.....	14	12	12	8	8	8	76	186	240	276	300	240	220	246	222	182	136	34	20	.....	.....	.....	.....
16	C	4	.....	.....	.....	.....	.....	.....	138	140	172	188	204	200	210	208	198	174	124	48	18	6	4	4	4
	D	3.2	.....	.....	.....	.....	.....	.....	158	178	190	194	240	236	254	258	224	186	140	48	20	14.2	3.4	3.4	3.4
18	C	16	.....	.....	.....	.....	.....	.....	.....	92	124	136	154	164	168	180	168	158	106	44	16	12	26	24	18
	D	12	.....	.....	.....	.....	.....	.....	.....	100	150	158	176	178	182	190	186	162	112	48	20	10	48	22	16
Average	.....	17.5	17.3	17.3	12.5	12.1	20.8	66.0	140	195	227.7	242	263	265.1	269.1	271	253.6	208.7	146.3	53.6	19.6	16.6	16.5	19.8	19.6
Percentage of maximum	.....	6	6	6	5	4	8	24	52	72	84	89	97	98	99	100	94	77	54	20	7	6	6	7	7

TABLE VI.—*Hourly solar radiation intensity (observed differential temperature) during oats transpiration period, at Akron, Colo., from August 4 to 18, 1912*

Date.	Hour ending— A.M. 6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6
Aug. 4	.....	.....	22.5	27.5	29	30	30	30	28.5	27	23	7	2
5	7	17	22	26	27	28	29	28.5	28	26	21.5	17	8
9	7	20	25	28	29.5	30.5	31	30	28.5	26	21.5	18	8.5
11	4	17	23	27	29	30	31	30	29	24	19.5	13	2
12	5	16	22	25	27	28.5	27	28	28.5	26.5	22.5	18	9
13	7	22	25	27	29	30	31	30.5	29	27	21	3	−1
16	7	20	25	27	29	30.5	31	30	29	30	22	17	8.5
18	9	17	25	28	20.5	31	31.5	31	29	27	21.5	17	8
Average	6.6	18.4	23.7	26.9	27.5	29.8	30.2	29.7	28.7	26.7	21.5	13.8	5.6
Calories per sq. cm. per minute	.22	.62	.79	.90	.92	1.00	1.01	1.00	.96	.89	.72	.46	.19
Percentage of maximum	22	61	78	89	91	99	100	98	95	88	71	46	19

TABLE VII.—*Hourly temperatures (in degrees Fahrenheit) during oats transpiration period, at Akron, Colo., from August 4 to 18, 1912*

Date.	Hour ending— A.M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8	9	10	11	12
Aug. 4	61.5	61.5	61.5	61.8	62	62	64	67.5	70	74	78	80.6	83	84	83	82.5	78	75	72	70	65	65	71	67
5	65	63	60	61.5	62	65	68	70	72	73	75	78	80	81.5	82	81	79	76	70	69	69	67	58	58
8	51.6	51	50	52	52	53	57	59	62	63	66	65	67	68	69	68.5	67	66	61	57	55	55	52	52
9	54	52	51	49.5	51	54	63	67	69	71	72.5	73	76	76	77	76	76	72	65	62	59	57	57	55
11	66	66	64	64	62	62	69	74	77	80	83	84	87	88	88	87	85	80	71	66	62	60	63	69
12	72	70	67	63	63.5	65	71	72	75	78	80	80.5	82	82.5	82	80	79	76	69	65	66	64	65	62
13	60	60	58	57.5	55	59	60	63	71	76	79	82	84	87	85	85	83	78	75	67	64	60	62	58
16	74	74	76	73	72	69	61	61	60	61	61	60	60	60	57	56.5	56.5	52	63	68	71	75	79	81
18	55	54	54	55	54	56	61	66	71	76	79	82	83	84	85	84	83	80	71	68	65	66	64	62
Average	62.1	61.3	60.2	59.7	59.3	60.6	63.8	66.6	69.7	72.4	74.9	76.2	78	79	78.7	78	76.3	72.9	69.6	65.8	64	63.2	63.4	62.7
Average in degrees centigrade	16.7	16.3	15.7	15.4	15.2	15.9	17.7	19.2	20.9	22.4	23.8	24.6	25.6	26.1	25.9	25.6	24.6	22.7	20.9	18.8	17.8	17.3	17.4	17.1
Percentage of maximum range	14	10	5	2	0	7	23	37	53	66	79	86	95	100	98	95	86	69	52	33	24	20	21	17

TABLE VIII.—*Hourly wet-bulb depression (in degrees Fahrenheit) during oats transpiration period at Akron, Colo., from August 4 to 18, 1912*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Aug. 4	1	1	1	1	1.5	1.5	2	2	4	6	8	9	10.5	12	12.5	13	13	12	9.5	7	5	4	8	7
5	8	9	7	5	4.5	4.5	4.5	6.5	9	10	10	10.5	12	14	14.5	14.5	13.5	12.5	8	6	4.5	4	6	4
8	.5	0	0	0	0	.5	.5	2	6	8.5	11	13	12.5	12.5	12	11	6	4.5	4	3	2.5	2	2	2
9	1.5	1	1	1.5	7	11	13	14	14	15	15	15.5	15	14.5	13	10	6	4.5	3.5	3	2.5	2	1.5	1.5
11	9	9	8.5	8.5	12	16	17	18	19.5	20	21.5	21	20	19	16	12	10	8	6	6.5	7	10	11	9.5
12	7.5	6.5	7	.....	.....	.....	9	15	18.5	18.5	20	21	20.5	21	20.5	20	19	18	14	11	9	10	8.5	8.5
13	7	6	6	5.5	5.5	5	4.5	5.5	11	14	17	18.5	19.5	20.5	20	19	18	16	11	9	8	5	5.5	6
16	3.5	3.5	3.5	3.5	3	3	3	3	4	6	10	15	20.5	20.5	23	20	20.5	20	16	12	9	7	5	4.5
18	4.5	4.5	4	3.5	3.5	3.5	3	3	5	8	14	17	19	19.5	21	22	22	21	17	12	9	8.5	9	10
Average	4.7	4.5	4.2	3.6	4.6	5.6	0.3	7.7	10.1	11.8	14.1	15.6	16.6	17.1	16.9	15.7	14.2	12.9	9.9	7.7	6.3	5.8	6.3	5.9
Percentage of maximum range	8	7	4	0	7	15	20	30	48	61	78	89	96	100	99	90	79	69	47	30	20	16	20	17
Saturation deficit...inches	0.140	0.125	0.119	0.095	0.131	0.141	0.189	0.220	0.308	0.392	0.461	0.529	0.584	0.602	0.592	0.555	0.488	0.418	0.305	0.217	0.193	0.177	0.196	0.167
Percentage of maximum	23	21	20	16	22	23	31	37	51	65	77	88	97	100	98	92	81	96	51	36	32	29	33	28

TABLE IX.—*Wind velocity (in miles per hour) during oats transpiration period at Akron, Colo., from August 4 to 18, 1912*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Aug. 4	10.5	10.2	11.1	7.6	8.3	9.0	7.5	8.0	7.0	7.3	8.5	8.6	8.6	10.2	10.8	10.5	7.8	3.9	7.9	7.2	6.4	8.6	16.5	15.4
5	12.1	7.6	6.1	7.0	6.8	5.9	.....	6.2	8.1	7.2	6.6	6.4	5.6	5.7	6.4	6.5	5.4	2.9	2.1	4.4	6.3	13.0	3.2	3.1
8	3.5	3.0	4.4	3.6	5.1	4.5	6.0	5.0	4.2	3.0	3.1	3.5	3.3	4.7	5.0	3.6	4.4	.35	.35	.45	.6	1.3	.3	.3
9	.3	1.05	.0	.0	.0	.0	2.5	2.5	3.0	3.5	3.1	4.7	4.2	5.1	5.4	4.8	5.5	4.4	2.0	1.1	.7	1.4	1.1	3.0
11	4.7	3.9	4.4	7.0	7.8	9.0	8.2	7.2	4.5	4.5	4.8	4.4	5.5	6.5	5.0	2.7	1.4	.6	.85	1.65	2.9	8.0	8.2	5.9
12	3.7	5.4	5.0	.....	.....	.....	.....	8.4	9.4	11.0	10.0	10.0	10.0	10.3	9.9	9.8	7.9	3.7	2.2	5.2	5.2	4.5	2.8	1.9
13	2.0	2.6	3.4	2.0	.7	2.2	7.3	6.7	6.1	6.1	6.1	4.7	5.5	8.5	9.1	7.9	6.6	4.4	6.1	9.3	6.0	7.0	3.9	.9
16	1.0	1.1	1.6	2.3	2.9	5.8	5.4	5.2	4.4	4.0	3.4	3.2	3.3	3.3	3.6	6.1	5.4	2.9	1.1	1.9	2.0	1.9	.5	.7
18	.2	1.9	1.0	.9	3.6	4.7	.....	5.1	3.8	2.8	2.9	3.8	5.1	5.3	5.1	5.9	5.5	2.1	.3	.3	2.5	.9	5.5	2.6
Average	4.2	4.1	4.1	3.4	4.4	5.1	6.2	6.0	5.6	5.5	5.4	5.5	5.7	6.6	6.7	6.4	5.5	2.8	2.5	3.5	3.6	5.2	4.7	3.8

The smoothness of the graphs obtained by this method of composites is in evidence in figure 4. The radiation curve is again seen to be symmetrical with reference to the noon hour and to decrease in either direction, at first slowly and then rapidly, to zero, a type of curve characteristic of clear days. The air temperature, wet-bulb depression, and transpiration all reach their maximum about two hours later. The transpiration graph for oats, like that for wheat, gives evidence of a slight depression or undue flattening after 9 a. m. In other words, one would expect from the corresponding slopes of the radiation and temperature curves that the transpiration graph would be more convex through the period from 9 a. m. to 2 p. m., provided the oat plant responds as a free physical system. It will be noted that the transpiration rate also falls off more rapidly in the afternoon than does the air temperature or the wet-bulb depression. In this respect the transpiration graph corresponds rather strikingly with the solar-radiation and wind-velocity graphs. The increase in wind velocity during the night does not, however, produce a corresponding increase in transpiration. This point will be referred to again. Finally, it is of interest to note that the transpiration loss of oats under Akron conditions during the night hours is extremely small, compared with the loss during the day.

SORGHUM

The sorghum transpiration measurements, like those made with wheat and oats, were conducted inside the screened inclosure and include three varieties of *Andropogon sorghum*—namely, Minnesota Amber, milo, and Dwarf milo [1] (Table X).

The environmental measurements for the corresponding period are given in Tables XI to XIV, inclusive.

[1] Minnesota Amber, A. D. I. 341–13; milo, S. P. I. No. 24960: Dwarf milo, S. P. I. No. 24970.

TABLE X.—*Transpiration rate (in grams per hour) of sorghum, at Akron, Colo., from August 23 to 29, 1912*

Variety.	Balance No.	Date.	Hour ending— A.M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8	9	10	11	12
Minnesota Amber	B	Aug. 23	10	10	10	10	10	10	72	170	220	340	400	420	460	440	400	340	320	120	54	34	20	20	20	14
Do	B	24	14	20	20	16	14	24	156	242	320	340	430	500	500	420	370	240	136	38	20	22	20	18	22	....
Do	B	25	22	20	20	20	18	28	80	120	200	150	216	160	210	264	260	230	110	28	28	32	28	24	16	20
Do	B	26	20	14	14	14	14	20	60	150	230	294	374	374	352	392	340	310	190	114	28	18	10	10	4	4
Do	B	27	4	4	4	4	12	30	100	180	210	460	440	500	520	530	470	370	244	136	54	40	34	20	20	20
Do	B	28	....	....	....	....	....	....	....	176	232	266	310	360	384	396	386	340	226	140	46	22	22	12	12	10
Do	B	29	10	10	....	....	....	....	56	108	212	324	392	436	456	434	380	296	192	78	44	34	14	24	22	12
Milo	C	25	18	18	18	10	14	16	100	140	180	280	....	....	400	440	480	400	320	130	36	26	28	26	22	20
Do	C	26	16	14	14	14	14	40	100	134	226	288	366	380	410	386	344	310	186	96	22	14	2	2	2	2
Do	C	27	2	2	2	2	2	36	100	180	240	360	380	520	520	510	460	350	310	90	46	32	30	20	18	16
Do	C	28	12	12	10	10	10	10	70	170	190	230	274	324	380	364	334	292	216	120	26	20	14	8	8	10
Do	C	29	12	4	4	4	4	4	32	200	210	280	300	360	440	440	390	370	270	50	36	40	16	18	12	4
Dwarf milo	D	24	16	14	14	14	10	16	52	110	150	210	250	....	....	340	300	260	210	110	20	14	12	12	10	16
Do	D	25	16	10	10	10	10	10	36	114	160	234	284	354	336	336	300	260	214	100	18	18	20	24	20	14
Do	D	27	2	2	2	2	2	8	50	130	190	260	320	340	380	400	332	264	186	70	44	30	24	20	14	12
Do	D	29	....	....	....	....	....	....	20	80	130	200	300	320	330	340	320	300	220	90	40	24	22	....	....	....
Average	....	....	12	11	11	10	10	20	72	150	206	282	336	382	405	402	368	307	222	94	35	26	20	17	15	12
Percentage of maximum	....	....	3	3	3	2	2	5	18	37	51	69	83	94	100	99	91	76	55	23	9	6	5	4	4	3

TABLE XI.—*Hourly solar radiation intensity (differential temperatures) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912*

Date.	Weight.	Hour ending— A.M. 6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6
Aug. 23	1	6	19	25	26	28	29	29.5	29.5	27.5	25	20	18	3
24	2	5	14	24	26.5	29	30	31	31	31	27	24	19	10
25	3	7	17	24	27	28.5	30	31	31	30.5	28	23	19	6
26	2	6	17	23	26	28.5	30	31	30.5	28	26	20	14	4
27	3	8	14	23	27	29.5	31	32	32	31	25	18	11	5
28	2	9	16	22	24.5	26.5	29	30.5	30	28.5	26	21	16	9
29	3	4	21	25	27.5	30.5	32	33	33	31.5	29	24	19	2
Average	....	6.5	16.8	23.7	26.5	29	30.4	31.4	31.2	30.1	27	21.6	16.4	5.5
Calories per sq. cm. per minute	....	.22	.56	.79	.89	.97	1.02	1.05	1.05	1.01	.90	.72	.56	.18
Percentage of maximum	....	21	54	75	84	92	97	100	99	96	86	69	53	18

TABLE XII.—*Hourly temperatures (in degrees Fahrenheit) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912* [1]

Date.	Weight.	Hour ending— A. M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7	8	9	10	11	12
Aug. 23	1	57	56	55	57	55	60	68	73	78	82	86	89	90	90	91	90	86	80	74	70	64	61	63	65
24	2	63	60	59	57	56	63	68	72	76	81	85	87	88	89	87	87	84	76	68	66	65	60	65	66
25	3	70	65	58	60	60	64	73	75	80	87	91	93	94	94	95	92	89	80	74	73	75	71	67	66
26	2	60	62	63	61	60	67	69	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	64	61.9	59.1	59.1	58.4	64	70.1	73.7	78.3	84.1	88.1	90.3	91.3	91.7	91.7	90	86.8	78.7	72	70.2	69.8	65.7	65.7	65.8
Average in degrees centigrade	.....	17.8	16.6	15.1	15.1	14.7	17.8	21.2	23.2	25.7	28.9	31.2	32.4	32.9	33.2	33.2	32.2	30.4	25.9	22.2	21.2	21	18.7	18.7	18.8
Percentage of maximum range	.....	17	11	2	2	0	17	35	46	60	77	89	96	99	100	100	95	85	61	41	35	34	22	22	22

[1] The thermograph record for this period is incomplete, but the days for which no record is given were similar in character to those recorded, as shown by the following maximum and minimum temperatures:

	Maximum. °F.	Minimum. °F.
Aug. 23	92	56
24	90	57
25	95	60
26	90	53
27	96	57
28	89	58
29	96	55

17208°—16——2

TABLE XIII.—*Hourly wet-bulb depression (in degrees Fahrenheit) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912*

Date.	Weight.	Hour ending— A.M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8	9	10	11	12
Aug. 23	1	5.5	5	5	4.5	4	4	4.5	4.5	9	13	16	17	19	20.5	22	23	25	26	22	18.5	16.5	13	11	10
24	2	10.5	11.5	12	10.5	9	8.5	8.5	10	14	17	20	23	27	28	28.5	28	27	25	22	18	13	11.5	11	10
25	3	11	11.5	12	11	9	8	7.5	8.5	16	19.5	23	27	31	31	31.5	30.5	31	31	23	17	16	16	17	16
26	2	13	12	10.5	10	11	11	11.5	14	19	22	25	25	24.5	24.5	22	21.5	19	17	12	9	6	5.5	4.5	3.5
27	3	2.5	1.5	1.5	3	4	4	8	12	16	19	23	26	27	27	27	27.5	26	26	21	16.5	14	14.5	14	11
28	2	8.5	7	5.5	5	5	5	6	10	15	18	20	22	23	23.5	23.5	24	22	20.5	18	14	10	9	7	5.5
29	3	5	5	4.5	3.5	2.5	1.5	1	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average		7.8	7.5	7.2	6.7	6.3	5.8	6.6	8.6	15.5	19.1	21.9	24.3	26.3	26.7	26.6	26.5	25.5	24.8	19.8	15.5	12.7	12.0	11.5	9.9
Percentage of maximum range		10	8	7	4	2	0	4	13	46	64	77	89	98	100	100	99	94	91	67	46	33	30	27	20
Saturation deficit, inches		0.222	0.206	0.179	0.167	0.179	0.178	0.218	0.294	0.565	0.750	0.909	1.020	1.107	1.138	1.138	1.110	1.004	0.815	0.576	0.471	0.393	0.333	0.321	0.287
Percentage of maximum		19	18	16	15	16	16	19	26	50	66	80	90	97	100	100	98	88	72	51	41	35	29	28	25

TABLE XIV.—*Wind velocity (in miles per hour) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912*

Date.	Weight.	Hour ending— A.M. 5	6	7	8	9	10	11	Noon. 12	P.M. 1	2	3	4	5	6	7	8
Aug. 23	1	5.0	5.3	7.6	10.3	8.9	8.3	7.4	6.1	5.7	5.4	6.2	5.8	5.4	1.0	1.3	1.1
24	2	5.4	5.3	6.7	8.3	8.2	6.7	8.1	8.2	7.3	4.5	3.6	3.4	5.0	3.9	.9	.5
25	3	6.2	5.6	7.1	10.1	10.0	8.5	8.7	7.1	5.1	6.1	3.4	6.1	5.0	1.2	.3	.5
26	2	6.7	7.2	7.0	3.8	4.1	3.5	4.6	4.6	6.5	7.2	9.6	9.4	9.8	5.8	3.0	2.5
27	3	1.3	6.0	7.0	9.0	9.8	10.2	10.8	10.7	10.0	10.0	9.4	6.6	6.5	10.5	5.8	3.4
28	2	3.5	4.5	5.3	6.9	2.3	10.3	8.4	7.3	5.4	4.9	5.4	6.1	6.0	5.7	2.4	1.4
29	3	2.5	3.8	3.0	7.2	9.8	9.4	10.6	10.2	10.6	7.7	7.1	8.7	9.2	8.5	4.6	5.7
Average		4.1	5.3	6.1	8.0	7.9	8.4	8.7	8.1	7.6	6.9	6.5	6.7	6.8	5.8	2.9	2.4

The sorghum measurements were made during the latter part and the oat measurements during the first part of August. The amplitude and spread of the radiation curves for the two periods are essentially the same (see figs. 4 and 5). The air temperature during the sorghum period was, however, much higher, the average daily maximum being over 91° F.,

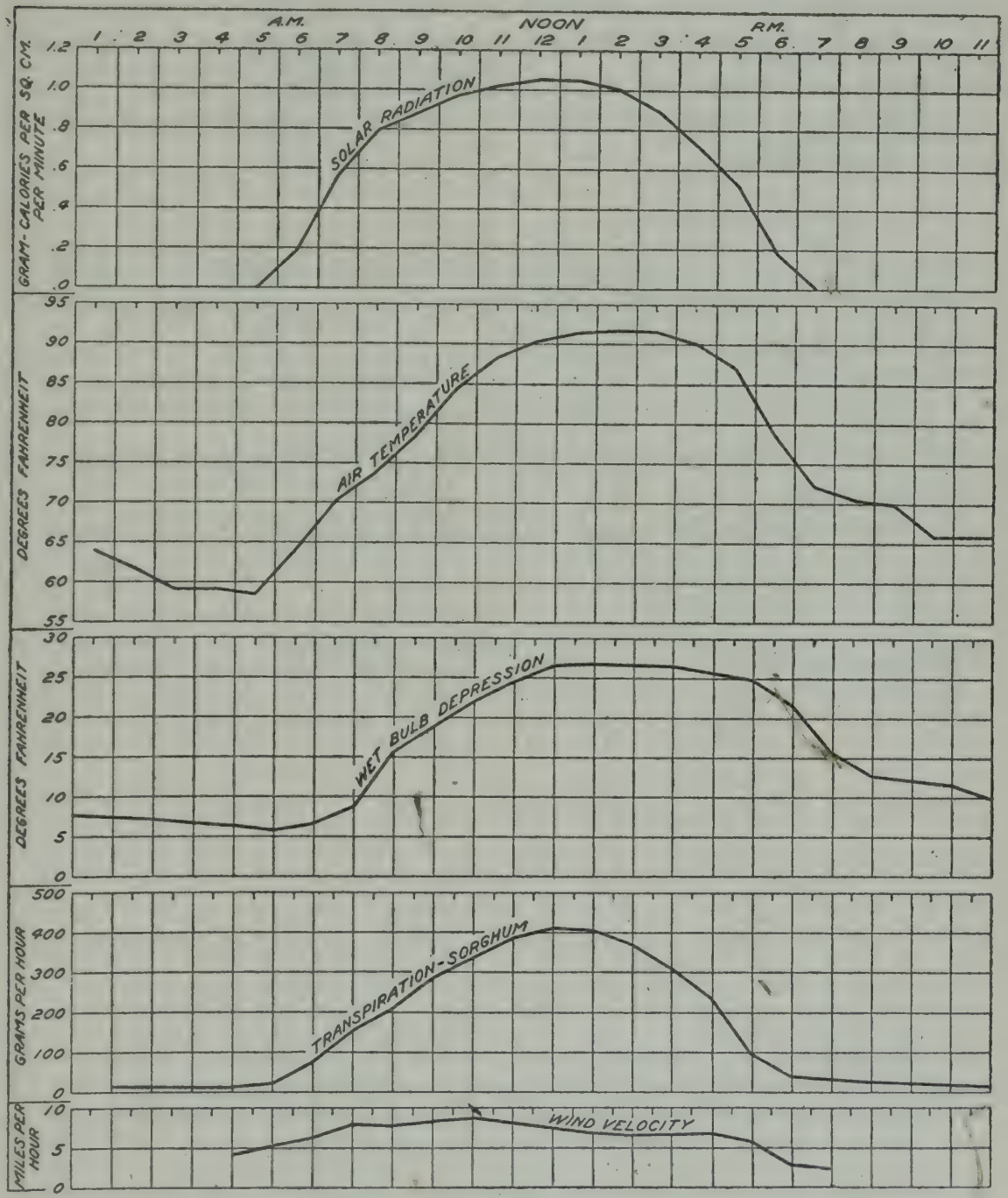

FIG. 5.—Composite transpiration graph of sorghum, with environmental graphs for corresponding period.

compared with a maximum of 79° during the oat period. There is also a corresponding difference in the wet-bulb depression, the mean maximum depression during the sorghum period being over 26°, compared with 17° during the oat period. The conditions were consequently more severe during the sorghum period—i. e., such as to induce a higher transpiration rate. Yet it will be seen, on reference to the transpiration graph

in figure 5, that sorghum, even under the more severe conditions imposed, gave no indication of a flattening of the peak of the transpiration curve. Furthermore, the maximum of the sorghum transpiration curve occurs at approximately noon, and the curve is nearly symmetrical. In brief, the transpiration graph of sorghum appears to follow more nearly the radiation curve than either wheat or oats. It is of interest in this connection to note that sorghum is one of the most efficient of the crop plants in the use of water, the sorghum varieties used in these experiments having a water requirement amounting to only 64 per cent of that of the oat plants.[1]

RYE

The transpiration data for rye (*Secale cereale*) [2] on clear days are given in Table XV. These observations were made outside the inclosure, under freely exposed conditions, from June 22 to July 3, 1914. The environmental measurements for this period are given in Tables XVI to XX, inclusive. Hourly evaporation measurements from a free-water surface were also made in 1914, with the aid of an automatic balance. The hourly means for the environmental factors are plotted in figure 6, together with the hourly evaporation and the hourly transpiration of rye, the latter being represented by the mean of 12 automatic records taken on six different days.

[1] Based upon water-requirement measurements of the same plants. (Briggs and Shantz, 1914.)
[2] Spring rye, C. I. No. 73.

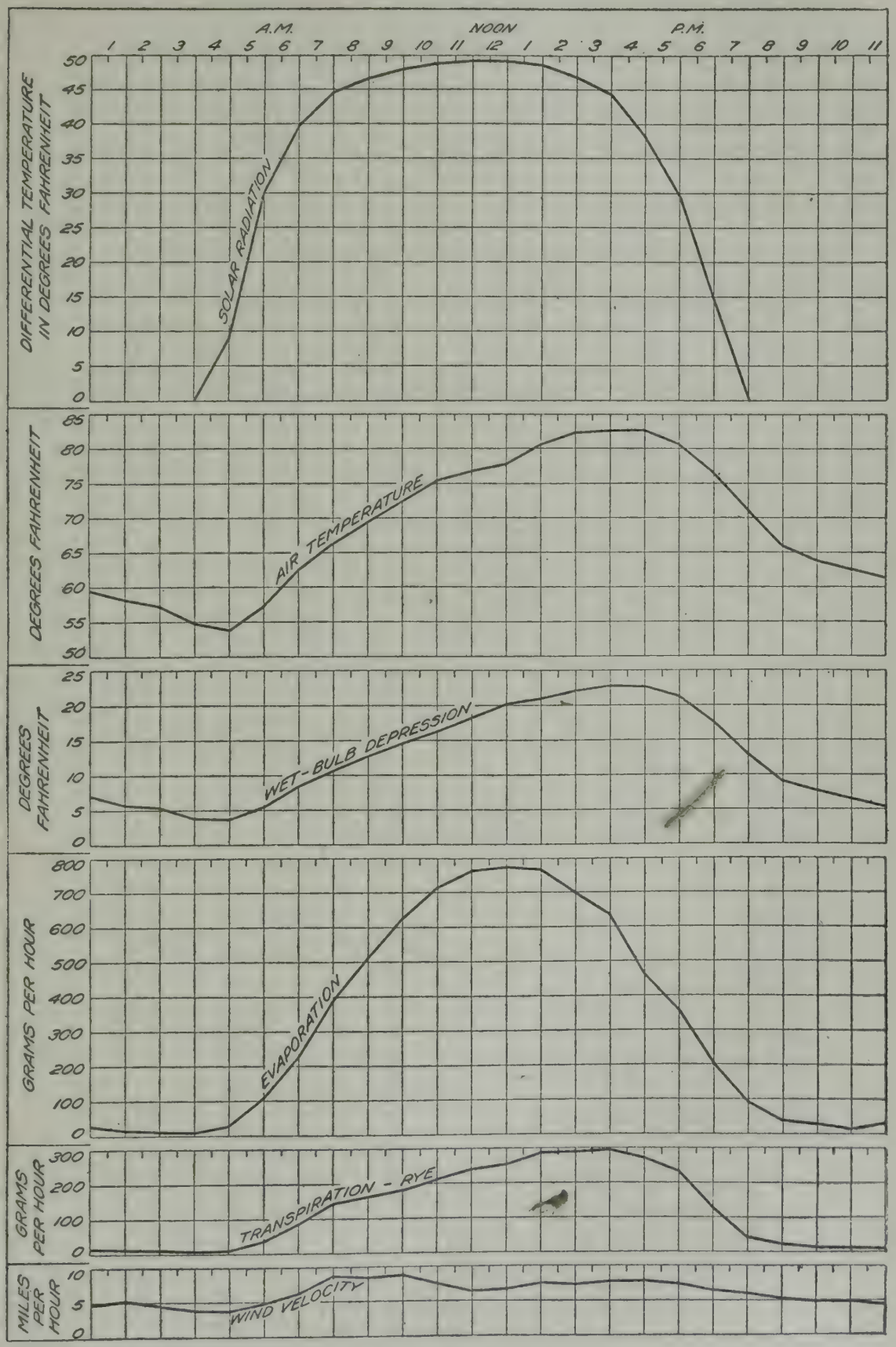
A.M.
NOON
P.M.
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11
DIFFERENTIAL TEMPERATURE IN DEGREES FAHRENHEIT
50 45 40 35 30 25 20 15 10 5 0
SOLAR RADIATION
DEGREES FAHRENHEIT
85 80 75 70 65 60 55 50
AIR TEMPERATURE
DEGREES FAHRENHEIT
25 20 15 10 5 0
WET-BULB DEPRESSION
GRAMS PER HOUR
800 700 600 500 400 300 200 100 0
EVAPORATION
GRAMS PER HOUR
300 200 100 0
TRANSPIRATION - RYE
MILES PER HOUR
10 5 0
WIND VELOCITY

FIG. 6.—Composite transpiration graph of rye, with environmental graphs and evaporation graph for corresponding period.

TABLE XV.—*Transpiration rate (in grams per hour) of rye, at Akron, Colo., during June and July, 1914*

Date.	Balance No.	Hour ending— A. M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7	8	9	10	11	12
June 22	A	.....	.....	.....	.....	20	80	140	190	190	220	220	260	260	280	320	300	300	230	70	46	30	16	12	12
22	C	.....	.....	.....	.....	0	100	130	200	210	200	210	230	240	300	300	300	300	260	74	30	10	20	20	10
24	A	30	40	60	0	0	16	66	190	190	180	200	260	320	360	380	400	400	350	210	46	10	10	10	10
24	C	20	30	30	0	0	16	66	140	160	200	200	260	300	320	340	380	360	300	200	60	0	0	0	16
27	A	0	0	0	0	20	20	100	130	160	184	210	200	210	230	240	280	240	240	160	40	30	20	20	20
27	C	16	6	0	0	6	56	100	128	156	184	210	200	200	240	240	260	240	240	148	40	20	20	20	20
29	A	20	0	0	20	10	10	40	100	120	140	180	180	230	290	250	240	240	220	140	40	40	10	10	20
29	C	20	20	16	16	20	20	80	140	140	150	150	240	240	260	280	280	250	250	140	40	20	20	20	10
July 2	A	0	0	0	20	10	26	66	140	190	210	240	260	260	300	260	240	220	140	60	20	.....	.....	.....	.....
2	C	16	20	10	10	20	50	100	150	190	210	240	240	240	280	280	260	200	150	60	20	.....	.....	.....	.....
3	A	.....	.....	.....	.....	6	36	60	100	150	170	260	280	280	280	300	290	250	220	100	60	20	20	10	10
3	C	.....	.....	.....	.....	0	16	60	120	140	200	260	260	260	280	290	290	260	200	120	46	36	20	20	0
Average	.....	16	14	14	8	10	38	84	144	166	187	214	240	254	286	290	294	272	234	124	42	22	16	14	12
Percentage of maximum	.....	5	5	5	3	3	13	29	49	56	64	73	82	86	97	99	100	93	80	42	14	7	5	5	4

TABLE XVI.—*Hourly solar radiation intensity (differential temperatures in degrees Fahrenheit) during rye transpiration period at Akron, Colo., in June and July, 1914*

Date.	Hour ending— A. M. 5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7
June 22	7	18	39	44	48	49.5	50	50	50	49	47	45	43	37	13
24	12	33	42	46	48	50.5	51	50	48.5	48	46	44	40	34	16
27	9	31	39	44	46	47	48	49	49	48	46	44	39	35	20
29	7	25	38	43	46	47	48	48.5	48.5	48	47	44	41	35	22
July 2	11	30	40	44	45	47	48	48.5	49	49.5	47	42	28	12	3
3	10	28	37	45	47	47	48	48.5	49	48	47	46	37	23	14
Average	9.3	27.5	39.2	44.3	46.7	48.0	48.8	49.1	49.0	48.4	46.7	44.2	38.0	29.3	14.7
Calories per sq. cm. per minute	0.26	0.80	1.10	1.24	1.31	1.34	1.37	1.38	1.37	1.36	1.31	1.24	1.06	0.82	0.41
Percentage of maximum	19	58	80	90	95	97	99	100	100	99	95	90	77	60	30

TABLE XVII.—*Hourly temperatures (in degrees Fahrenheit) during rye transpiration period at Akron, Colo., during June and July, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 22	64	60	60	57.5	56.5	58.5	61.0	65.0	68.0	69.5	71.0	73.0	74.5	76	78	78.5	78.5	75.5	71	66	63.0	63.5	64.0	63
24	61	61	62	59.0	57.5	61.5	67.5	70.5	72.0	74.5	78.5	81.5	83.0	84	86	86	86.5	84.0	78	73	69.0	67.5	64.5	63
27	56	55	54	50.0	49.5	55.0	62.0	65.0	68.5	71.5	75.0	77.0	79 0	81	81.5	82	84.0	82.5	77	68	61.5	59.5	58.5	56
29	58	55	50	48.0	47.5	53.0	59.0	62.0	66.5	70.0	73.0	71.0	79.0	81	81.5	81.5	81.0	79.5	76	71	68	64.0	64.5	64
July 2	57	56.5	56.5	56	56.5	58.5	63.0	67.5	71	73.5	76	78	81	81.5	82	82.5	82	81	79	74	65	62	60	60.5
3	60	61	61	58	56	57	62.5	67	70	75	78.5	80.5	82.5	83	84	84.0	83.5	81.5	77.5	74	69	66	64	61.0
Average	59.3	58.1	57.2	54.8	53.9	57.2	62.5	66.2	69.3	72.3	75.3	76.8	79.8	81.1	82.2	82.4	82.6	80.7	76.4	71.0	65.9	63.8	62.6	61.2
Average in degrees centigrade	15.2	14.5	14.0	12.7	12.2	14.0	16.9	19.0	20.7	22.4	24.1	24.9	26.6	27.3	27.9	28.0	28.1	27.1	24.7	21.7	18.8	17.7	17.0	16.2
Percentage of maximum range	19	15	12	3	0	12	30	43	54	64	75	80	90	95	99	99	100	93	78	60	42	35	30	25

TABLE XVIII.—*Hourly wet-bulb depression (in degrees Fahrenheit) during rye transpiration period at Akron, Colo., in June and July, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 22	.....	.....	.....	.....	7	9	9.5	9	11	12	12.5	14	15	16	18	19.5	19.5	19	16	11	7	5	5.5	.5
24	7	4	5	3	1	1.5	4	7.5	8	8	10	13	15	16.5	18.5	21	21.5	19.5	15	10	6	5	3.5	3
27	12	11	10	7	7	9	12	15	17	20	22	23	24.5	25	26.5	27	27	24	18	13	10	9.5	9.5	7.5
29	7	6	5	4	2	3	8	10	12	12	14	17	20	21	22	22.5	22	21	18	16	14	10	7	6
July 2	5	4.5	4	3.5	5	7	10	14	17	18	19	21	23	22	23	23	22	21	18	11	5	5	5	4
3	4	3	3	2	1	3	7	8	12	17	19	21	23	24	24	24	24	23	20	16	12	11	8	7
Average	7.0	5.7	5.4	3.9	3.8	5.4	8.4	10.6	12.8	14.5	16.1	18.2	20.1	20.8	22.0	22.8	22.7	21.2	17.5	12.8	9.0	7.6	6.4	4.7
Percentage of maximum range	17	10	8	1	0	8	24	36	47	56	65	76	86	90	96	100	100	92	73	47	27	20	14	5
Saturation deficit, inches	0.180	0.131	0.135	0.095	0.093	0.134	0.265	0.288	0.367	0.443	0.528	0.586	0.683	0.733	0.788	0.820	0.826	0.724	0.686	0.397	0.249	0.204	0.194	0.123
Percentage of maximum	22	16	16	12	11	16	32	35	44	54	64	71	83	89	95	99	100	88	83	48	30	25	23	15

TABLE XIX.—*Wind velocity (in miles per hour) during rye transpiration period at Akron, Colo., in June and July, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 22	1	3	2	3	2	3.8	5	11	9	7	4	3.5	3.8	7	8	11	11	11	8	2	2	4.5	6.5	6.5
24	2	4.5	9.5	6.5	5.5	6.3	5	4.3	6	6.5	5.5	4.5	5.5	6	6	7.5	8	9.5	10	5.6	3.3	2.6	2.3	1.5
27	7.5	8.5	7.0	4.0	3.0	3	4	6.5	8.5	8.0	7.0	4.5	5.5	3.3	3.3	3	2.5	1	2	4.5	3	3	3	1.6
29	5	3	0.3	0.3	0.3	1	5	7	7.5	6.5	7.5	8	8.5	10.5	10	10.5	11	10.5	10	8	8	5	6	8
July 2	6	6	6	6	9	10	13	16	13	13	11.0	9	9	10	9	7	6	5	2.5	13	9	9	7	6
3	6.5	6	2.5	3.5	3.5	5.5	4.5	7.5	7	11	10.0	9	7	8	7	7	8	8	6	3	5	5	4.5	4
Average	4.7	5.2	4.5	3.9	3.9	4.9	6.1	8.7	8.5	8.7	7.5	6.4	6.6	7.5	7.2	7.7	7.8	7.5	6.4	6.2	5.1	4.9	4.9	4.6

TABLE XX.—*Evaporation rate (in grams per hour) during rye transpiration period at Akron, Colo., in June and July, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 22	20	20	20	20	20	212	368	420	460	520	600	620	700	700	680	610	530	360	120	16	26	20	40	0
24	60	60	60	40	40	30	70	360	500	540	640	720	740	760	720	620	520	420	260	20	0	0	0	0
27	0	0	0	0	46	106	240	480	540	580	790	790	760	700	600	620	360	360	320	160	0	0	0	96
29	60	0	0	0	0	60	240	360	400	560	660	780	820	840	720	680	500	420	250	170	120	46	36	50
July 2	10	10	0	0	46	94	220	440	580	720	780	820	680	800	720	600	380	230	130	60	0	0	0	0
3	0	0	0	0	10	140	220	280	600	840	820	840	900	800	760	700	480	360	140	130	90	110	20	60
Average	25	15	14	10	27	107	226	390	514	626	715	762	767	767	700	638	462	358	203	93	39	29	16	34
Percentage of maximum	3	2	2	1	4	14	29	51	67	82	93	99	100	100	91	83	60	47	26	12	5	4	2	4

A striking feature of the radiation curve is the rapid rise in radiation intensity during the early morning hours. Reference to the graphs will show that the radiation has attained approximately one-half its maximum value two hours after sunrise, and a corresponding decrease occurs in the late afternoon.

The mean air temperature during the rye transpiration period ranged from 54° F. at 4.30 a. m. to about 83° F. at 4.30 p. m. The maximum air temperature thus occurs four hours later than the solar-radiation maximum. The wet-bulb-depression graph is similar in form to the air-temperature curve, and its maximum occurs at approximately the same time. The maximum of the evaporation curve, on the other hand, corresponds with that of solar radiation, but the slope of the evaporation graph is more nearly uniform during the morning and afternoon than that of the radiation graph.

The transpiration graph of rye shows the same flattening during the middle part of the day that was observed with wheat and oats in 1912. With rye this flattening begins at 8.30 a. m., and continues until 1 p. m., the slope being nearly uniform during this period. During the late afternoon the transpiration falls rapidly and the night transpiration is seen to be very low.

The mean wind velocity in miles per hour is plotted at the bottom of figure 6. The maximum rate of about 9 miles per hour occurs from 8 to 10 o'clock in the morning. During the night the rate is less than 5 miles per hour. There is little indication from the graphs that differences in the velocity of the wind had much influence on either the transpiration or the evaporation rate.

ALFALFA

The transpiration measurements upon alfalfa (*Medicago sativa*)[1] are the most extensive of the series and include 52 day records taken during 26 days, embracing late-season as well as midsummer measurements. The transpiration data are given in detail in Table XXI and the physical measurements in Table XXII to XXVI, inclusive. The hourly means will be found plotted in figure 7. Since the period covered by the measurements is so extended, it has seemed advisable also to separate the measurements into shorter periods for comparison. Summaries covering a short transpiration period in June and another period in October are accordingly presented in Tables XXVII and XXVIII, and are plotted in figure 15, to which reference will be made later.

[1] Grimm alfalfa, A. D. I. (Alkali and Drought Resistant Plant Investigations) No. 23.

TABLE XXI.—*Transpiration rate (in grams per hour) of alfalfa at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Balance No.	Hour ending— A. M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7	8	9	10	11	12
1913.																									
July 11	A	0	0	0	0	60	90	40	110	160	180	160	120	180	220	180	200	190	160	100	30	4	4	4	4
	C	30	20	0	0	20	60	70	90	150	180	200	150	160	200	200	220	160	150	90	4	4	4	4	4
12	A	0	0	0	0	0	20	160	200	220	220	220	200	220	240	260	280	240	220	140	50	10	0	0	0
	C	6	6	8	12	12	20	140	160	180	200	190	180	220	220	240	260	220	200	120	30	10	10	10	4
Aug. 10	A	4	4	4	4	40	20	20	120	200	240	320	280	360	380	400	400	340	260	100	30	4	4	4	4
	C	2	2	2	2	2	20	80	180	240	320	320	340	360	400	420	400	340	280	80	20	14	4	4	4
14	A	4	4	4	4	4	20	120	260	300	240	380	460	500	540	600	580	560	440	100	20	10	10	10	10
	C	4	4	4	4	4	20	140	320	400	440	460	520	600	600	600	600	600	440	120	20	20	10	10	10
1914.																									
June 18	A	8	20	20	20	30	70	260	240	200	200	280	280	380	320	340	360	340	240	140	6	6	6	6	10
	C	0	20	0	20	10	70	200	200	200	260	280	280	316	324	260	290	250	240	124	36	0	6	6	10
19	A	10	10	10	10	10	20	190	220	280	320	360	380	440	460	440	440	340	220	160	160	20	20	0	0
	C	10	0	0	0	0	70	150	220	260	280	300	320	340	380	380	360	350	310	70	70	20	0	0	0
21	A	0	0	0	0	10	20	90	220	260	320	400	430	430	460	440	400	400	300	120	66	14	4	0	0
	C	0	0	0	0	40	32	152	260	300	400	460	500	520	540	520	460	500	360	160	46	10	26	0	0
Aug. 11	A	16	52	80	32	20	160	200	160	300	320	300	440	560	600	600	560	520	420	160	30	34	20	20	32
	C	20	20	66	20	34	70	160	120	300	280	360	450	510	520	550	510	480	404	152	32	20	18	18	22
Sept. 10	A	20	0	0	0	0	10	70	330	420	420	460	560	580	580	590	524	479	216	36	10	6	6	6	6
	C	20	0	0	0	0	6	54	280	360	380	440	500	530	560	540	490	450	202	38	20	0	0	0	0
18	A	20	34	16	0	0	20	220	460	620	740	780	820	840	820	800	760	500	190	30	60	0	0	0	0
	C	16	24	0	0	0	0	160	460	560	620	680	720	740	760	710	690	500	188	22	40	10	0	0	0
19	A	0	0	0	0	0	0	124	356	540	640	660	680	820	800	760	640	530	220	30	10	10	10	10	6
	C	10	20	10	0	0	0	110	340	450	550	620	660	680	700	670	620	460	200	20	20	10	10	4	6
20	A	6	6	34	6	0	0	40	320	490	660	740	740	780	750	710	600	428	54	40	20	0	0	0	0
	C	6	24	10	10	0	0	50	280	400	550	590	620	680	660	660	590	400	60	40	20	0	0	0	0
23	A	6	6	26	34	20	8	120	120	260	350	430	470	454	406	340	360	240	104	32	32	14	20	10	14
	C	0	0	0	20	10	10	88	52	180	228	282	290	320	320	300	340	180	90	24	16	10	14	6	0
24	A	12	34	0	0	0	44	116	280	360	420	480	510	480	440	380	380	310	100	34	28	26	12	32	16
	C	10	10	10	10	16	4	80	220	250	330	360	440	410	430	400	360	260	100	20	10	10	16	20	20
25	A	40	30	16	14	20	20	120	380	440	580	620	660	710	720	670	620	332	180	28	30	24	36	10	24
	C	20	14	10	16	10	34	150	290	360	440	480	520	540	560	540	500	320	84	36	16	24	10	10	20
26	A	14	46	10	16	14	8	100	342	436	514	600	700	780	760	760	610	470	136	20	34	24	46	30	20
	C	16	14	10	20	0	10	100	240	320	340	440	500	520	580	540	500	360	128	24	22	16	30	6	6
30	A	6	6	6	20	40	20	120	260	460	640	720	800	840	700	700	680	370	74	12	14	14	26	40	0
	C	10	6	20	16	10	20	80	240	400	510	550	620	640	600	600	520	340	40	20	10	10	0	0	0
Oct. 5	A	0	0	0	0	0	0	72	220	344	420	500	540	560	560	560	500	400	100	60	40	10	10	20	0
	C	10	20	6	14	6	14	60	150	230	330	370	360	340	400	360	360	240	100	20	40	6	6	6	0

6.....	A	0	0	0	26	14	20	100	90	230	380	400	440	400	380	360	420	220	20	20	12	8	6	6	2
	C	0	0	0	20	0	0	14	66	160	240	300	380	380	420	400	400	260	40	10	10	10	10	10	10
14.....	A	0	0	0	0	0	0	10	60	70	140	130	100	200	220	160	170	110	40	14	16	30	10	10	0
	C	.....	.....	.....	.....	.....	.....	4	16	50	80	70	120	200	180	180	180	160	20	0	0	0	0	0	0
15.....	A	16	4	0	20	20	20	10	10	60	240	260	380	300	300	300	380	120	24	16	16	24	0	0	0
	C	0	0	20	0	20	20	6	14	160	220	240	320	290	290	260	260	140	50	10	0	0	0	20	20
16.....	A	40	0	0	0	0	60	54	186	240	260	360	340	360	380	340	320	80	80	16	34	40	30	0	40
	C	0	0	0	0	0	20	20	120	200	220	280	300	320	320	310	320	150	40	14	18	18	10	16	64
17.....	A	40	0	0	0	20	20	40	160	320	470	470	540	560	570	490	430	230	30	10	20	74	26	16	44
	C	10	10	16	10	6	6	20	130	250	360	360	440	460	480	460	380	210	40	30	30	20	24	14	10
18.....	A	0	0	20	20	20	40	60	220	360	440	540	600	580	560	440	400	220	40	10	10	60	80	20	20
	C	.....	.....	.....	.....	.....	.....	20	160	240	340	360	460	440	420	420	370	190	48	8	22	46	20	26	14
19.....	A	0	0	0	0	0	0	34	186	300	430	480	490	520	500	480	400	210	50	26	14	30	30	8	8
	C	0	0	0	0	0	0	16	124	208	308	400	440	440	440	400	350	170	34	16	20	10	14	8	10
20.....	A	24	14	6	6	6	24	54	246	340	480	610	610	690	630	600	460	220	60	16	34	10	10	10	16
	C	14	12	12	10	16	20	20	150	230	400	460	460	510	500	490	370	174	24	8	24	8	8	14	10
Average.....		10	10	9	9	12	25	90	204	293	367	414	451	481	482	464	434	313	151	53	27	16	13	10	10
Percentage of maximum.....		2	2	2	2	2	5	19	42	61	76	86	94	100	100	96	90	65	31	11	6	3	3	2	2

TABLE XXII.—*Hourly solar radiation intensity (differential temperatures in degrees Fahrenheit) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Hour ending—														
	A. M.							Noon.	P. M.						
	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7
1913.															
July 11	6	19	31	37	40	43	44	45	45	45	40	37	36	31	20
12	8	15	30	36	39	41	43	44	45	44·5	43	41	38	31	29
Aug. 10	0	11	22	35	39	41	43	43	43·5	43	41·5	39	36	28	10
14	4	17	32	37	40	42	45	46	46	45·5	44	36	36	27	7
1914.															
June 18	14	32	40	45	46	47	48	48	48	46	43	41	37	26	10
19	20	34	41	45	46	46	48	48	48	47	46	42	37	24	5
21	2	21	37	42	45	46	47	48	45	47	44	37	38	36	21
Aug. 11	3	12	36	44	48	49	50	51	51	50	49	45	40	32	14
Sept. 10	2	5	28	37	42	44	45·7	46	48·8	45·5	44	41	35	22	2
18	0	6	29	38	42	44·5	45·5	45·5	45	44	42	39	33	28	0
19	0	5	28	39	42	43·5	45	45	45	44	42	38	31	14	0
20	0	4	28	39	42	43·5	44·5	45·5	45	43	42	38	28	0	0
23	0	12	34	44	47	48	48	48·5	47	46	43	39	32	12	0
24	0	10	35	42	45	46	47	47	46·5	45	42	38	31	13	0
25	0	3	26	38	42	43·5	44·5	45	44·5	43	42	38	.....	.....	.....
26	0	6	29	39	43	45	45·5	46	45	43	41·5	37	29	10	0
30	0	4	24	37	41	42·5	44	45	44·5	43	41	38	29	7	0
Oct. 5	0	0	29	42	45	46	46·5	45	45·5	44	42	39	31	10	0
6	0	3	35	43	45	47	47	46·5	45	43·5	41·5	38	28	4	0
14	0	0	23	42	46	48	48	48·5	47	45	42·5	38	30	18	0
15	0	0	22	40	44	45	45	45	44	42·5	40	36	26	2	0
16	0	0	26	40	44	44	44	44·5	43·5	42	39	33	18	0	0
17	0	0	22	38	41	42	43	43	42	40·5	39	32	18	0	0
18	0	0	14	38	42	43	43	43·5	42·5	40	37	29	9	0	0
19	0	0	22	38	40	42	42·5	42·5	42	40·5	38	33	19	0	0
20	0	0	21	37	41·5	41·5	41·5	42	41·5	40	37	33	19	0	0
Average	2·3	8·4	28·6	39·7	43	44·4	45·3	45·6	45·2	44	41·8	37·5	29·7	15·1	4·7
Percentage of maximum	5	18	63	87	94	97	99	100	99	97	92	82	65	33	10
Calories per sq. cm. per minute	.06	.24	.80	1.11	1.20	1.24	1.27	1.28	1.27	1.23	1.17	1.05	.83	.42	.13

TABLE XXIII.—*Hourly temperatures (in degrees Fahrenheit) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1913.																								
July 11	76	69	61.5	59 5	58	57.5	57.5	59	64	67	71	73	75.5	76	76.5	76.5	76.5	75.5	74	71.5	65	61.5	57	54
12	50	49.5	48.5	47.5	49	53	59	66	71	74	77	80	83	85	87.5	88	88.5	87.5	86	80	73	68	68	69
Aug. 10	65.5	65	64	60.5	59	59	60.5	65	70	73	76	78	80	82	83.5	84	84	83.5	71	67	73	71	68	65
14	63	62	60	59.5	58.5	58.5	63	69	73	78	83	86	89	91	91	92	91.5	91	86	80	76	74	71	69
1914.																								
June 18	59	59	58	58	59.5	60	64	68	72.5	77.5	82	84	86	88	88.5	89	89	88	84	75	68	65	64	65
19	65	62	61.5	61	59	58.5	64	73.5	79	82	85	88	89	90	90.5	90.5	89.5	88	83	77.5	75	73	68	65
21	61	59	58	56	54	57.5	62.5	67.5	72	75.5	81	83.5	85	87	88	86	86	86	82	76	76	75	67	65
Aug. 11	60	60.5	61.5	60	58	54	54	58	62	67	70	73.5	77	79	80	80.5	80	79	75	67	62	60	60	60
Sept. 10	55.5	55	52.5	52	51	52	55	60	68	71	74	77	79	80.5	81	80.5	80	78	69	63	58	55.5	53	52.5
18	61	63.5	62.5	59	58	60	71	80	84	86	87.5	88	88.5	88	87	85	82	76	71	70	68	66	64.5	63.5
19	62.5	61.5	61	60.5	59.5	60	65	72	78	80	83.5	84	85	85.5	84.5	83	80	74	68	65	63	63	61	60
20	58.5	58	57	56	54	54.5	63	69	74	79.5	82	84	86	86.5	86	84	79	73	73	71	69	68.5	67	64
23	42.5	41	41.5	41	39	38	44	52	58	62	65	67	68	69	70	70.5	68.5	63	55.5	52	46	42.5	41.5	43
24	45	44	43.5	40	39.5	43.5	50	62	67.5	70.5	73	75	76.5	77.5	78	78	77	71	61	55	52	51	48	50.5
25	54.5	54.5	54	53	52.5	51.5	56	63	71	75.5	77.5	80	81	81.5	81.5	81	79	74	67	63.5	61.5	61	49	49
26	57.5	57	55.5	63	49.5	47.5	49	57	67	71	78	81	84	85	85	85	83	77.5	68	62.5	56.5	58.5	58	55.5
30	47	46	47	48	47.5	47	51	62	72	76	78	80	80.5	80.5	80.5	80	78	72	64	60	57	55	54	52
Oct. 5	34	33	34	34	34.5	35	37	43	53	57	60	62	64.5	66	66.5	66	64	58	51	51	47	45	43.5	43
6	37	34	31	29.5	30	31	34	42	54	62.5	66	69	71	71.5	71.5	71	68	61	54	51	50	47	42	42
14	23	22.5	24	24.5	24	24	27	34	42	47	51	53	55	58	59.5	59	57	52	44	37	31	30	32	33.5
15	33	32	31	31	32.5	33	36	44	51.5	56.5	62	64	66.5	68	69	68	64	59	51	46	40.5	38.5	36	35.5
16	36	35.5	36	33.5	33.5	33.5	37	44	54	59	65	69	71	73.5	74.5	75	73	64	53	51	48.5	47	48.5	46
17	45	44.5	44.5	44.5	44	44	42.5	48	61	69	73	76	78.5	80	80.5	80	77	65	55	52.5	54.5	55	51.5	50.5
18	47	40.5	42.5	41.5	38.5	39.5	40	53	60	65.5	70.5	73	74.5	75.5	75.5	74.5	70	54	51.5	50	48	48.5	48	48.5
19	44	41	39	38.5	38	36.5	37	49	62	67	70.5	72	73.5	74.5	74	73	69	60	53	50	48.5	47	47	46
20	44.5	45.5	45	44.5	42.5	40	40	53	67	72	75.5	78	80	81	80.5	78	72	64.5	57.5	56	54.5	52.5	51.5	50.5
Average	51	49.8	49	48.3	47	47.2	50.7	58.2	65.7	69.7	73.8	76.1	77.9	79.2	79.7	79.1	77	72	65.6	61.6	58.5	56.8	54.6	53.8
Average in degrees centigrade	10.6	9.9	9.4	9.1	8.3	8.4	10.4	14.6	18.7	20.9	23.2	24.5	25.5	26.2	26.5	26.2	25	22.2	18.7	16.4	14.7	13.8	12.6	12.1
Percentage of maximum range	12	9	6	4	0	1	11	34	57	69	82	89	95	98	100	98	92	76	57	45	35	30	23	21

TABLE XXIV.—*Hourly wet-bulb depression (in degrees Fahrenheit) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1913.																								
July 11	10	4.5	3.5	4	4.5	5.2	8	12	14	16	19	20	21	21	21	20.5	20	19.5	17	14.5	12	10	9	7.5
12	7	6	5.5	6	7	13	18	20	21.5	23	24.5	26	28	29	29.5	28	28	27	24	18	15	14	13	11
Aug. 10	6.5	5.5	4	3	2.5	4	6	8.5	10.5	13	14.5	16.5	19	20	22	22	22.5	21	18	14	11	8	6	5
14	5	4	3	2.5	2	2	5	9	12	16	17	20	22	24.5	25	26	25	24	18	15	13	11.5	10	9
1914.																								
June 18	.....	.....	.....	.....	4.5	5	8	10	14	16	20	21.5	24	26	27	25.5	24	20	15	7	5.5	6	8	9
19	6	4.5	4.5	4	3	6	10	12.5	13	19	24	25	25	25	24	23	22	18	13	12	14	9	7	6
21	4	4	4.5	4.5	2.5	4	8	9	10	13	19	21	22	25	26	25	25.5	24	17	16	17	15	9	2
Aug. 11	8.5	7.5	6	4.5	4	1.5	3	5.5	8	10.5	13.5	18.5	21	23	24	24.5	25	24.5	19	14	13	10.5	8.5	7
Sept. 10	2.5	1	.5	0	0	0	.5	5	13.5	17.5	23.5	26	26.5	27.5	27	26.5	25.5	22.5	16	13	10.5	8.5	7.5	6.5
18	6	6	6	5	4	4	8	16	22	27	29.5	30	29	29	28.5	27	26	22	17	14	10	8	6	5.5
19	4.5	3.5	2	2	2	1	2	8	13	17	21	23	23	24	24	23	21	19	17	15	12.5	10	8	6
20	5	4	3	3	1.5	5	2	7.5	12.5	17.5	21	21.5	24	25	25	24.5	23	18	15	15	13.5	12	12	11
23	4.5	3	4	2.5	2	1	3	7	10	13	15	16	17	17	18	18	19	14	10.5	8	7	4	4	3.5
24	4.5	4.5	4	2.5	1	2	5	11	15	18	20	22	23	24	25	25	24	20	15	11	9.5	8.5	7.5	8.5
25	9	10	10	9.5	8.5	8.5	10	15	20	23	26	27	28	28	28	27	25	22	17	14	12	12	11	11
26	10.5	10	9	7	5	4	5	10	16	18	22	24.5	27	28	28.5	27	26	23	16	14	11	11	13	12
30	7	6	6	7	8	7	5	11	16	23	23.5	26	28	28	28	24	19.5	19	17	14	12	10	9	7
Oct. 5	1	1	1	1	1	1	3	7	13	18	19.5	20	22.5	23.5	25	24	22	17	13	12	9	7.5	6.5	5.5
6	3.5	2.5	2	1	5	1	1	6	11	15	19	21	24	24.5	25	24.5	22	16	12	10	8	7	4.5	4
14	3	1.5	3	3.5	.....	.....	11	8	6	7	9	11	12.5	14.5	16.5	17	15	12	9	5	2.5	1	1	2
15	2.5	1.5	.5	0	.5	1	3	8	11	15	18	19	21	22	22.5	22	18	14	12	10	6	4	2.5	2
16	2	2	2	1	1	1	1	6	11	15	19	21	23	23	24	25	23	19	13	12	10.5	10	10	8.5
17	7.5	7	7	7.5	7.5	7	3	7	13	21	23	25	27	28	28.5	28.5	26	19	14	12	12	13	11	10
18	10	5	5	6	3	3	5	13	16	20	22	23.5	23.5	25	24.5	25	22	16	13	12	11	10.5	10.5	10.5
19	9	5	3	1.5	1	.5	1	7	14	17	20	21.5	22	22	22	21	17	13	9	7.5	7.5	6	6.5	6
20	6	5	6	5.5	3.5	2.5	3	10	15	21	25	27	29	30	30	28	24	18	13	10	8	6	5	4
Average	5.8	4.6	4.2	3.8	3.2	3.6	5.3	9.6	13.5	17.3	20.3	22.1	23.6	24.5	25.0	24.3	22.7	19.3	15.0	12.3	10.5	9.0	7.9	6.9
Percentage of maximum range	12	6	5	3	0	2	10	29	47	65	78	87	94	98	100	97	89	74	54	42	33	27	22	17
Saturation deficit in inches	0.126	0.092	0.091	0.087	0.066	0.075	0.107	0.226	0.372	0.495	0.605	0.685	0.754	0.794	0.827	0.794	0.715	0.572	0.389	0.308	0.246	0.218	0.193	0.161
Percentage of maximum	15	11	11	11	8	9	13	27	45	60	73	83	91	96	100	96	86	69	47	37	30	26	23	19

TABLE XXV.—*Wind velocity (in miles per hour) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1913.																								
July 11	10	10	7	7	9	13	12	11	9	9	11	11	10	8	7	11	7	7	8	6	4	2	0	0
12	.5	.5	1	1	3	4	11	12	15	16	15	14	12	8	9	8	9	7	7	3	3	5	8	8
Aug. 10									12	11	10	10	8	8	7	7	6	7	5	3	4	4	.5	.5
14	1	1	2	1	2	1	1	7	5	5	3	2	3	3	3	5	8	7	3	1	.3	.3	.3	2
1914.																								
June 18	2	2	3	5	4	5	8	10	10	8	7	7	6	7	5	2	3	2	2	1	.5	.5	.5	.5
19	1	2	2	3	2	3	4	4	3	4	8	8	8	7	6	8	10	7	7	3	8	4	2	2
21								7	7	8	6	6	7	5	5	8	9	3	2	3	4	5	5	8
Aug. 11	9.5	10	8.8	7.3	6.0	6.7	8.5	10.3	11	9.6	8.2	8.1	8.2	6.5	5.9	4.4	5.3	5.2	2.8	1.5	1.9	2.5	3.9	4.1
Sept. 10	4	1.7	1.6	1.1	1.9	4.1	4.4	7.2	6.5	10.2	10.8	9.5	8	7.5	6.2	7.1	6	3.7	3.4	3.2	1.1	1.4	1.7	1.8
18	4	5.4	6	6.3	5	4.8	5.1	5.8	7.4	7.9	7.5	7.2	8.6	9.1	8.5	8.6	7.7	8.5	5.5	8.2	9.7	7.4	8	9.5
19	9.2	8.3	6.5	4.5	3.2	3.5	4.8	6.7	8.7	10	10	7.8	8.8	7.2	7.8	8.0	7.8	6.2	5.3	5.3	6.6	1.0	9.2	8.8
20	8	8.6	9	8.5	5.5	5.5	7.2	10.9	9.5	11	12	12.8	11.1	11	10.5	10	8.5	3.8	7.3	10	7	9.6	6	.....
23	3.9	3.4	5.2	5	6.3	7.2	9.8	4.6	3.8	4.8	5.5	6.1	7.6	7.1	4.9	6.5	7.5	3.1	3.3	1.8	1.2	2.3	2.7	3.0
24	2.9	4.0	3.7	2.1	2.5	4.3	4.1	5.2	2.6	3.1	4.0	5.7	4.2	3.6	3.4	3.2	1.5	1.6	1	1	2.2	3.1	3.7	4.7
25	5.2	6.1	7.3	5.9	5	5.1	5.2	7.5	8.1	9.7	10.6	10.4	8.8	8.5	7.8	8.3	8.3	5.2	4.0	4.3	6.1	7.5	8.2	9.4
26	8.8	9.6	8.8	5.9	3.3	3.0	3.8	5.2	7.1	5.2	3.1	3.4	5.4	5.1	5.9	5.0	4.4	2.6	2.1	2.4	2.8	3.9	5.0	5.3
30	2.2	2.3	2.5	4.3	6.5	5.5	4.6	4.4	4.7	10.6	11	10.3	9	8.9	8.2	.....	.....	.....	.....	.....	.....	.....	.....	.....
Oct. 5	4.1	4.5	6.8	5.0	5.0	4.8	4.9	6.8	11	14.5	13.5	14.5	13.6	14.0	12.3	11.5	9.3	5.0	4.6	5.2	4	2.1	2.2	1.9
6	1.2	1.5	1.5	2.4	3.3	5.5	9	9.2	4.6	2.0	2.4	3.1	3.8	4.8	6.4	8.4	8.2	5.4	4.1	2.3	1.8	1.4	1.1	1.1
14	2.6	1	1	.9	.8	.3	.2	1.2	10.9	11.9	9.8	6.2	5.9	4.9	6	5.9	4.1	1.5	1.6	1.2	2.1	2.6	3.5	4.0
15	4.6	5.4	6.9	6.8	8	7	7.5	6.2	7.3	9.1	10	10.4	11.4	11.6	10.5	10.8	7.9	1.4	2.6	1.5	1.5	2.2	2.2	2.8
16	4.7	5	4.7	2.7	2.6	4.7	8.9	12.0	12.0	14	10	7.8	4.3	4.3	2.5	2.9	7.4	2.5	2.6	3.2	4.6	5.7	5.1	5.9
17	8	7.8	7	7.8	6.5	5.2	6.6	5.4	3.9	6.2	4.7	4	6	5.3	5.5	6.3	4.8	2.3	3.6	4.6	4.5	5.4	4.1	5.1
18	5.4	4	6.1	6.5	5.3	10	2.6	6.2	7.3	5.3	10.7	11.8	7.1	5.3	4.8	3.9	3.2	3.4	2.9	1.8	3	4.6	4.7	4.7
19	3.2	2.5	2.8	3.3	3	2.4	1.9	1.7	3.4	4.1	5.5	5.8	7.0	7.0	6.8	6.5	3.8	1.9	1.2	2.3	3.2	3.9	4.5	3.8
20	4.9	4.4	3.9	3.4	2.6	2.1	2.9	3.5	2	4.3	3.4	8.6	8.6	8.3	9.7	10.8	8.6	6.4	4.6	6.7	8.5	7.5	9.5	9.9
Average	4.6	4.6	4.8	4.4	4.3	4.9	5.8	6.8	7.4	8.3	8.2	8.1	7.8	7.2	6.8	7.1	6.7	4.4	3.9	3.5	3.6	3.8	4.1	4.5

TABLE XXVI.—*Evaporation rate (in grams per hour) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1913.																								
July 11	0	0	0	0	.....	.....	.....	.....	380	600	.....	560	640	700	840	660	400	300	300	120	80	20	12	10
12	20	20	20	40	20	60	360	400	580	1,040	740	760	860	780	740	640	520	440	280	180	80	120	160	100
Aug. 10	10	10	6	6	6	80	140	340	480	580	780	760	700	800	780	600	520	360	120	80	40	22	2	2
14	2	2	2	2	2	20	160	400	540	440	620	680	680	740	580	520	520	380	120	60	20	30	40	50
1914.																								
June 18	0	0	0	0	40	180	360	440	600	560	920	860	900	900	520	530	440	220	160	20	0	10	10	40
19	20	10	10	0	20	100	200	340	520	700	820	940	920	880	720	700	640	400	200	140	60	0	0	0
21	0	0	0	0	20	20	140	340	500	640	800	800	780	780	760	660	560	280	80	140	180	200	20	140
Aug. 11	94	88	112	20	72	210	384	340	360	300	432	760	780	660	660	480	400	280	90	60	50	30	46	32
Sept. 10	0	0	0	0	0	8	62	480	510	680	680	720	720	680	590	536	334	150	150	70	30	40	12	38
18	34	36	0	0	0	4	108	248	460	620	700	840	880	780	720	640	460	266	160	120	70	40	50	40
19	40	20	0	0	0	0	68	212	400	620	780	780	760	700	680	500	400	260	180	140	120	120	94	60
20	50	30	36	16	34	0	70	250	420	650	760	850	940	760	700	590	450	200	140	40	10	10	30	0
23	10	16	34	50	0	0	100	120	260	460	640	680	680	540	480	500	200	60	80	20	40	40	40	20
24	0	0	0	0	0	60	80	240	290	480	630	680	600	560	480	388	232	110	60	40	30	66	80	54
25	80	60	90	38	52	0	132	288	420	660	850	890	860	740	688	592	400	220	160	110	110	148	140	166
26	146	140	154	106	40	0	100	140	420	440	590	640	720	680	600	480	300	160	100	100	70	60	52	76
30	32	28	32	20	20	0	20	140	340	800	840	860	840	720	660	540	340	200	120	110	90	100	60	30
Oct. 5	0	0	0	0	0	0	14	92	194	550	670	700	660	640	600	430	324	126	80	80	40	20	0	0
6	0	0	0	0	0	0	48	44	168	340	440	560	540	480	480	420	280	120	30	40	24	12	6	36
14	0	0	0	0	0	0	8	112	234	280	320	340	400	380	400	260	80	0	0	0	0	0	0	0
15	20	50	50	30	150	40	40	100	60	180	580	620	660	540	500	400	.....	.....	.....	.....	.....	.....	.....	.....
16	.....	.....	.....	.....	.....	.....	80	140	260	520	620	530	470	360	240	200	160	100	40	40	40	40	40	40
17	.....	.....	.....	.....	.....	.....	140	180	200	240	400	600	700	600	540	460	280	120	180	110	24	148	86	130
18	30	34	56	90	56	44	100	214	406	480	700	700	460	540	400	320	160	116	44	60	116	140	100	80
19	0	0	0	0	0	0	0	68	232	370	520	550	600	520	440	352	208	70	30	40	60	52	28	12
20	28	20	60	20	20	20	50	150	180	360	620	700	700	700	620	480	320	180	90	70	50	70	14	20
Average	26	24	28	19	24	37	118	232	362	524	659	706	710	660	594	496	357	204	120	80	57	62	45	47
Percentage of maximum	4	3	4	3	3	5	17	33	51	74	93	99	100	93	84	70	50	29	17	11	8	9	6	7

TABLE XXVII.—*Summary of transpiration and environmental conditions during alfalfa transpiration period at Akron, Colo., from June 18 to 21, 1914*

Physical condition.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Transpiration:																								
Average	5	8	5	8	17	47	174	227	250	297	347	365	404	414	397	385	363	278	129	64	12	10	2	3
Percentage of maximum	1	2	1	2	4	11	42	55	60	72	84	88	98	100	96	93	88	67	31	15	3	2	0	1
Evaporation:																								
Average	6	3	3	0	26	100	233	373	540	633	847	867	867	853	667	630	547	300	147	100	80	70	10	60
Percentage of maximum	0	0	0	0	3	12	27	43	62	73	98	100	100	98	77	73	63	35	17	12	9	8	1	7
Radiation:																								
Average	.....	.....	.....	.....	12	29	39.3	44	45.7	46.3	47.7	48.0	47.0	46.7	44.3	40.0	37.3	28.7	12.0	.....	.....	.....	.....	.....
Percentage of maximum	.....	.....	.....	.....	25	60	82	92	95	96	100	100	98	97	92	83	78	60	25	.....	.....	.....	.....	.....
Calories per sq. cm. per minute	.....	.....	.....	.....	0.34	0.81	1.10	1.23	1.28	1.30	1.34	1.35	1.32	1.31	1.24	1.12	1.05	0.80	0.34	.....	.....	.....	.....	.....
Air temperature:																								
Average	61.6	60.0	59.1	58.3	57.5	58.6	63.5	69.6	74.5	78.3	82.6	85.1	86.7	88.3	89.0	88.5	88.2	87.3	83.0	76.2	73.0	71.0	66.3	65.0
Average in degrees centigrade	16.4	15.6	15.1	14.6	14.2	14.8	17.5	20.9	23.6	25.7	28.1	29.5	30.4	31.3	31.7	31.4	31.2	30.7	28.3	24.6	22.8	21.7	19.1	18.3
Percentage of maximum range	13	8	5	3	0	3	19	38	54	66	80	88	93	98	100	98	97	95	81	59	49	43	28	24
Wet-bulb depression:																								
Average	5.0	4.3	4.3	4.3	3.3	5.0	8.7	10.5	12.3	16.0	21.0	22.5	23.7	25.3	25.7	24.5	23.8	20.7	15.0	11.7	12.2	10.0	8.0	5.7
Percentage of maximum range	8	4	5	4	0	8	24	32	40	57	79	86	91	98	100	95	92	78	52	38	40	30	21	11
Saturation deficit:																								
Average	.136	.115	.125	.131	.087	.137	.143	.326	.420	.571	.776	.863	.935	1.018	1.043	.996	.970	.847	.591	.430	.399	.325	.239	.174
Percentage of maximum	13	11	12	13	8	13	14	31	40	55	74	83	90	98	100	96	93	81	57	41	38	31	23	17
Wind velocity	1.5	2.0	2.5	4.0	3.0	4.0	6.0	7.0	6.7	6.7	7.0	7.0	7.0	6.3	5.3	6.0	7.3	4.0	3.7	2.3	4.2	3.2	2.5	3.5

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TABLE XXVIII.—*Summary of transpiration and environmental conditions during alfalfa transpiration period, at Akron, Colo., from October 16 to 20, 1914*

Physical condition.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Transpiration:																								
Average	14	4	6	5	8	21	34	168	269	371	432	468	488	480	443	380	185	45	15	23	32	25	13	24
Percentage of maximum	3	1	1	1	2	4	7	34	55	76	89	96	100	98	91	78	38	9	3	5	7	5	3	5
Evaporation:																								
Average	19	18	39	37	25	21	74	150	256	394	572	616	586	544	448	362	226	117	77	64	58	90	54	56
Percentage of maximum	3	3	6	6	4	3	12	24	42	64	93	100	95	88	73	59	37	19	13	10	9	15	9	9
Radiation:																								
Average	.....	.....	.....	.....	.....	0	21.0	38.2	41.7	42.5	42.8	43.1	42.3	40.6	38.0	32.0	16.6	0	.....	.....	.....	.....	.....	.....
Percentage of maximum	.....	.....	.....	.....	.....	0	49	89	97	99	99	100	98	94	88	74	39	0	.....	.....	.....	.....	.....	.....
Calories per sq. cm. per minute	.....	.....	.....	.....	.....	0	0.59	1.07	1.17	1.19	1.20	1.21	1.18	1.14	1.06	0.90	0.46	0	.....	.....	.....	.....	.....	.....
Air temperature:																								
Average	40.3	41.4	41.4	40.5	39.3	38.7	39.3	49.4	60.8	66.5	70.9	73.6	75.5	76.9	77.0	76.1	72.2	61.5	54.0	51.8	50.8	50.0	49.3	48.3
Average in degrees centigrade	4.6	5.2	5.2	4.7	4.1	3.7	4.1	9.7	16.0	19.2	21.6	23.1	24.2	24.9	25.0	24.5	22.3	16.4	12.2	11.0	10.4	10.0	9.6	9.1
Percentage of maximum range	4	7	7	5	2	0	2	28	58	73	84	91	96	100	100	98	88	60	40	34	32	30	28	25
Wet-bulb depression:																								
Average	6.9	4.8	4.6	4.3	3.2	2.8	2.6	8.6	13.8	18.8	21.8	23.6	24.9	25.6	25.8	25.5	22.4	17	12.4	10.7	10.0	9.1	8.6	7.8
Percentage of maximum range	17	9	9	7	3	1	0	26	48	70	83	91	96	99	100	99	85	62	42	35	32	28	26	22
Saturation deficit:																								
Average	.129	.090	.086	.081	.061	.054	.053	.184	.348	.508	.627	.704	.745	.783	.809	.785	.662	415	.278	.235	.214	.197	.185	.164
Percentage of maximum	16	11	11	10	8	7	7	23	43	63	77	87	92	96	100	97	82	51	34	29	26	24	23	20
Wind velocity	5.2	4.7	4.9	4.7	4.0	4.9	4.6	5.8	5.7	6.8	6.9	7.6	6.6	6.4	5.9	6.8	5.6	3.3	3.0	3.7	4.8	5.4	5.6	5.9

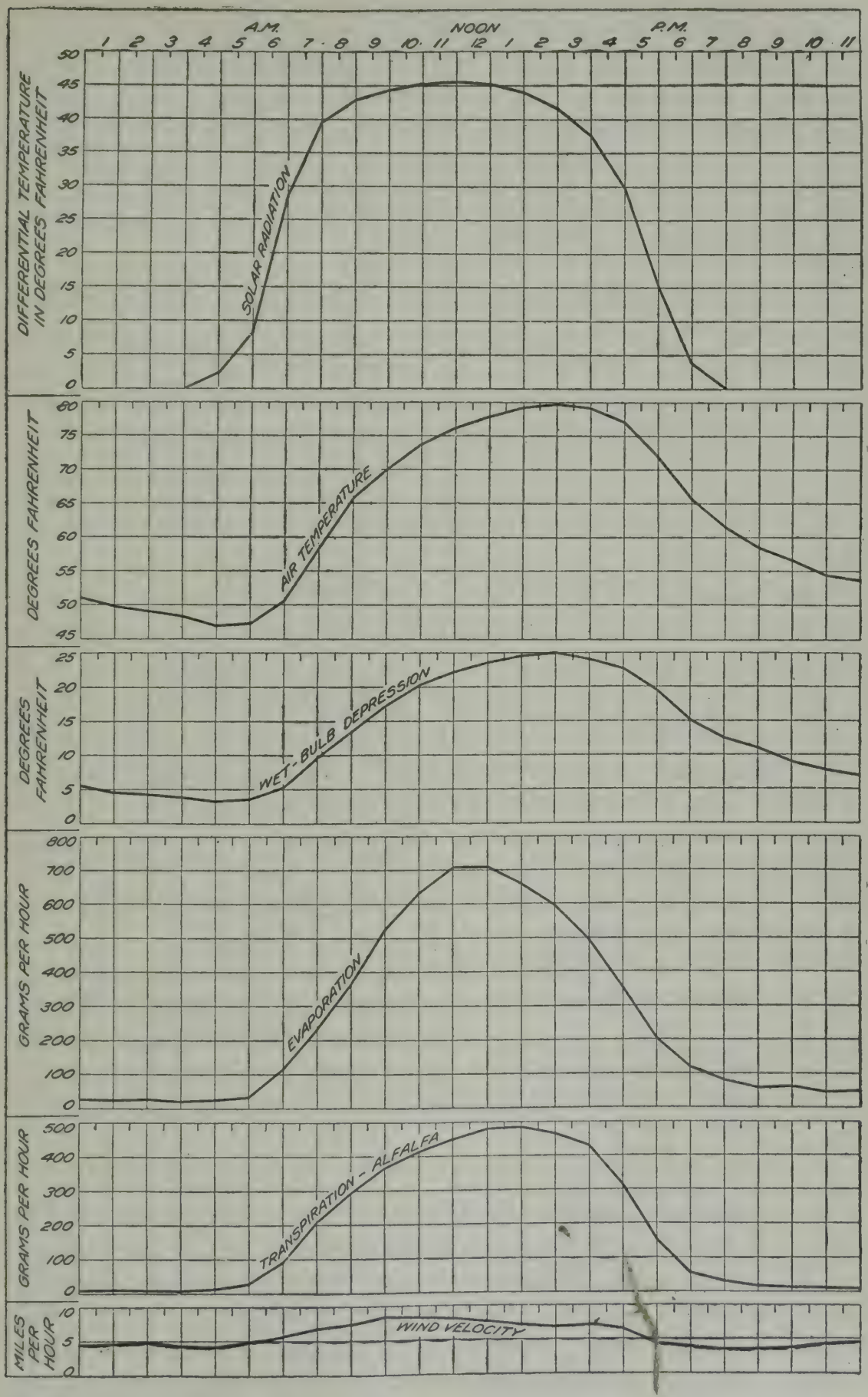
A.M.
NOON
P.M.
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11
DIFFERENTIAL TEMPERATURE IN DEGREES FAHRENHEIT
50 45 40 35 30 25 20 15 10 5 0
SOLAR RADIATION
DEGREES FAHRENHEIT
60 75 70 65 60 55 50 45
AIR TEMPERATURE
DEGREES FAHRENHEIT
25 20 15 10 5 0
WET-BULB DEPRESSION
GRAMS PER HOUR
800 700 600 500 400 300 200 100 0
EVAPORATION
GRAMS PER HOUR
500 400 300 200 100 0
TRANSPIRATION - ALFALFA
MILES PER HOUR
10 5 0
WIND VELOCITY

FIG. 7.—Composite transpiration graph of alfalfa, with environmental graphs and evaporation graph for corresponding period.

Considering now the composite graphs based upon the records obtained during 26 clear days, it will be seen that the radiation graph is similar in form to those already discussed, save that the radiation tends to change less rapidly during the early-morning and late-afternoon hours, owing to the fact that the length of the day was not uniform throughout this long period. The slight variation in radiation intensity during the midday hours and the marked changes between 5 and 7 a. m. and 4 and 6 p. m. are in conformity with what has already been noted of the other radiation curves.

The composite temperature graph shows a daily range of 33 degrees, the minimum (47° F.) occurring between 4 and 5 a. m., and the maximum (80° F.) between 2 and 3 p. m. The graph showing the wet-bulb depression is very similar in form to the air-temperature graph, and the maxima and minima correspond. This is to be expected, since with an unvarying amount of water vapor in the air, the wet-bulb depression would be determined by temperature fluctuations. Furthermore, since the observations are confined to clear days, sudden changes in absolute humidity are not encountered.

The evaporation graph representing the alfalfa period is nearly symmetrical with respect to noon, and the slope of the graph changes but slightly during either the morning or afternoon hours. The greater portion of the daily evaporation, however, takes place during the afternoon, owing probably to the higher temperature prevailing during this part of the day.

The transpiration graph shows a very low rate of transpiration during the night. The rate gradually increases from about one hour after sunrise to the maximum at 1.30 p. m. After 2.30 p. m. the curve falls rapidly until sundown and remains practically constant throughout the night. By far the greater part of the daily transpiration occurs during the afternoon. This asymmetry with respect to midday is much more apparent in the transpiration graph than in the evaporation graph.

At the bottom of figure 7 the mean velocity of the wind is shown for each hour in the day. During daylight hours the rate is approximately 7 miles per hour and during the night about 4 miles per hour. It is apparent from Table XXIV that the air is never still for an hour at a time.

AMARANTHUS

The transpiration data so far presented have been confined to crop plants. It is also desirable in this connection to study the transpiration of weeds or native plants which have shown themselves adapted to regions of limited rainfall. To this end, *Amaranthus retroflexus* was selected as a plant widely distributed throughout the cultivated areas of the United States. Amaranthus is also one of the most efficient plants known as regards the use of water, its water requirement at Akron being below 300, thus comparing favorably with the best of the prosos, millets, and sorghums, the most efficient crop plants known.

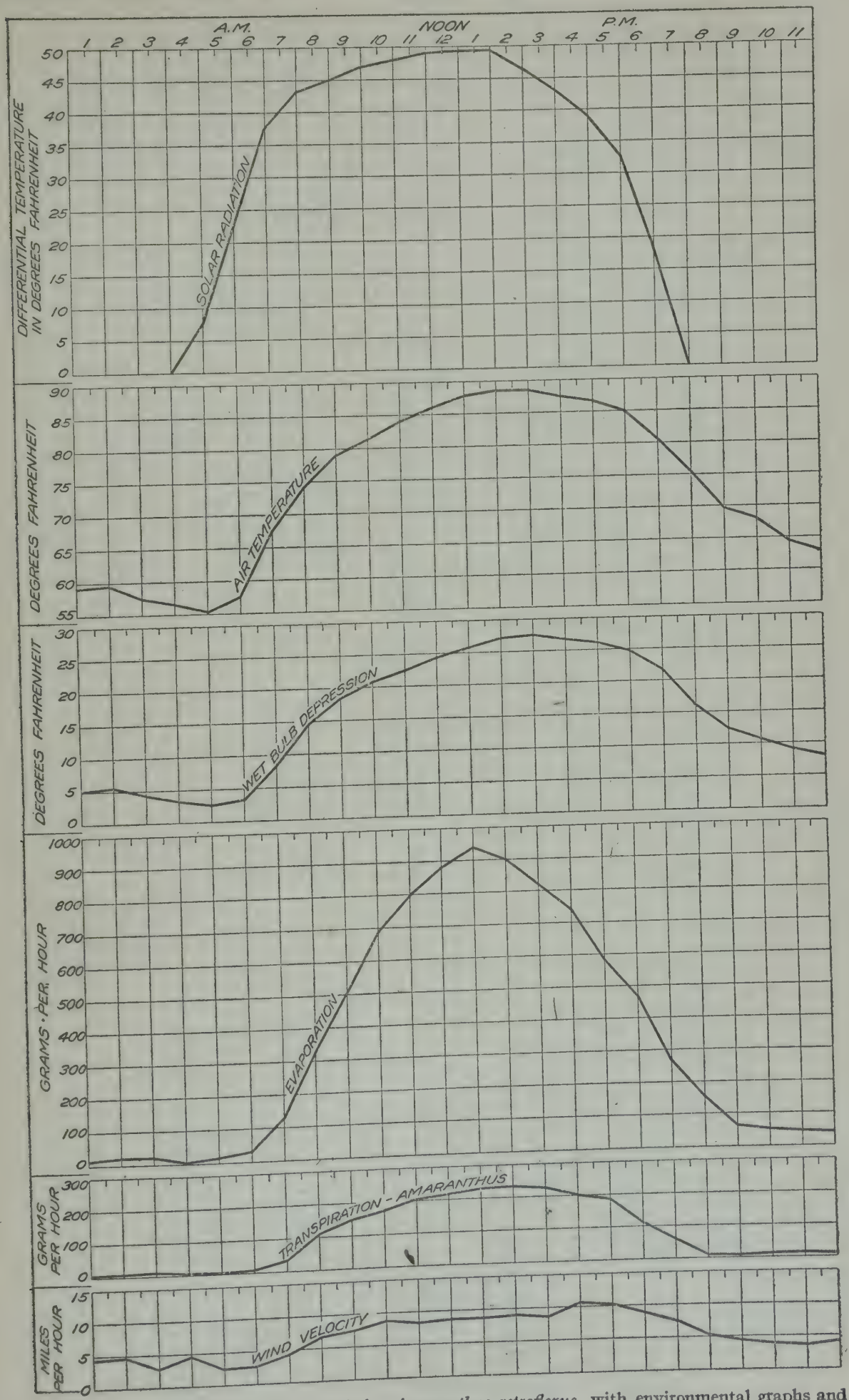
A.M.
NOON
P.M.
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11
DIFFERENTIAL TEMPERATURE IN DEGREES FAHRENHEIT
50 45 40 35 30 25 20 15 10 5 0
SOLAR RADIATION
DEGREES FAHRENHEIT
90 85 80 75 70 65 60 55
AIR TEMPERATURE
DEGREES FAHRENHEIT
30 25 20 15 10 5 0
WET BULB DEPRESSION
GRAMS PER HOUR
1000 900 800 700 600 500 400 300 200 100 0
EVAPORATION
GRAMS PER HOUR
300 200 100 0
TRANSPIRATION - AMARANTHUS
MILES PER HOUR
15 10 5 0
WIND VELOCITY

FIG. 8.—Composite transpiration graph for *Amaranthus retroflexus*, with environmental graphs and evaporation graph for corresponding period.

TABLE XXIX.—*Transpiration rate (in grams per hour) of Amaranthus retroflexus at Akron, Colo., from July 7 to 9, 1914*

Date.	Balance No.	Hour ending— A. M. 1	2	3	4	5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7	8	9	10	11	12
July 7	A	0	0	0	20	0	0	15	65	80	90	150	140	168	192	180	140	120	104	96	0	0	0	0	0
	C	0	0	0	0	0	0	16	74	90	100	180	180	210	220	200	180	140	110	92	16	12	0	0	0
8	A	0	0	0	0	6	8	40	140	140	180	210	230	220	220	220	240	220	100	26	6	6	20	10	10
	C	0	20	20	0	6	10	46	140	200	240	220	280	260	260	270	250	240	140	62	8	8	4	4	4
9	A	10	10	20	0	0	10	26	94	200	200	200	200	220	280	250	170	180	100	40	5	5	5	5	20
	C	4	4	20	0	0	10	50	160	200	230	250	260	280	230	220	220	230	120	44	6	6	24	50	24
Average		2	6	10	3	2	6	32	112	152	173	202	215	226	234	223	200	188	112	60	7	6	9	12	10
Percentage of maximum		1	3	4	1	1	3	14	48	65	74	86	92	97	100	95	86	80	48	26	3	3	4	5	4

TABLE XXX.—*Hourly solar radiation intensity (differential temperatures in degrees Fahrenheit) during Amaranthus retroflexus transpiration period at Akron, Colo., from July 7 to 9, 1914*

Date.	Hour ending— A. M. 5	6	7	8	9	10	11	Noon. 12	P. M. 1	2	3	4	5	6	7
July 7	4.0	16	35	42	45	46	47	47	48	50	49	41	34	29	21
8	9.0	23	36	44	44	47	49.0	51	51	49	43	43	42	35	14
9	10.0	29	40	44	45	47	49.0	49	49	49	47	45	41	34	20
Average	7.7	22.7	37	43.3	44.7	46.7	48.3	49.0	49.3	49.3	46.3	43.0	39.0	32.7	18.3
Calories per sq. cm. per minute	0.21	0.64	1.04	1.21	1.26	1.32	1.34	1.37	1.38	1.38	1.30	1.20	1.09	0.92	0.51
Percentage of maximum	16	46	75	88	91	95	98	99	100	100	94	87	79	66	37

TABLE XXXI.—*Hourly temperatures (in degrees Fahrenheit) during Amaranthus transpiration period at Akron, Colo., from July 7 to 9, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
July 7	60	59	57.5	57.5	56	57	63	68	72	74	78	81	83	84	84.5	84	83	81.5	78	73	69	67	63	60
8	58	60	56	54	53	58	70	77	82	85	87	89	91	92	91.5	90.5	89	88	83	77	71	70	65	62
9	60	60	59	58	57	58	70	78	83	85	87	88	89	89.5	89.5	89	88	86	81	76	68	64	65	66
Average	59.2	59.6	57.4	56.5	55.3	57.6	67.7	74.3	79.0	81.3	84.0	86.0	87.7	88.5	88.5	87.8	86.6	85.2	80.6	75.3	69.3	67.0	64.3	62.7
Average in degrees centigrade	15.1	15.3	14.1	13.6	12.9	14.2	19.8	23.5	26.1	27.4	28.9	30.0	30.9	31.4	31.4	31.0	30.3	29.4	27.0	24.1	20.7	19.4	17.9	17.1
Percentage of maximum range	12	13	6	4	0	7	37	57	71	78	87	93	98	100	100	97	94	90	76	60	42	35	27	22

TABLE XXXII.—*Hourly wet-bulb depression (in degrees Fahrenheit) during Amaranthus retroflexus transpiration period at Akron, Colo., from July 7 to 9, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
July 7	4	4	2.5	3.0	1.0	0.5	4	8	10	11	14	17	19	21	22	22	20	20	18	13	10	8	6	4
8	3	4	4.0	2.0	2.0	3.0	8	17	21	25	26	27	28	29	29	27	28	26	23	17	14	13	11	10
9	8	8	6.0	5.0	5.0	6.0	13	19	24	26	27	29	30	31	31	31	30	28	24	18	13	11	10	10
Average	5.0	5.3	4.2	3.3	2.7	3.2	8.3	14.7	18.3	20.7	22.3	24.3	25.7	27.0	27.3	26.7	26	24.7	21.7	16.0	12.3	10.7	9.0	8.0
Percentage of maximum range	9	11	6	2	0	2	23	49	63	73	80	88	94	99	100	98	95	89	77	54	39	33	26	22
Saturation deficit, inches	0.126	0.137	0.097	0.076	0.067	0.078	0.238	0.498	0.621	0.723	0.823	0.919	0.988	1.051	1.056	1.013	0.973	0.909	0.746	0.516	0.375	0.306	0.245	0.215
Percentage of maximum	12	13	9	7	6	7	23	47	59	68	78	78	94	100	100	96	92	86	71	49	35	29	23	20

TABLE XXXIII.—*Wind velocity (in miles per hour) during Amaranthus retroflexus transpiration period at Akron, Colo., from July 7 to 9, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
July 7	9	3.8	3	6	3	3	4.7	6.5	8	8	7.5	9	8.5	9	8	9	8	8	8	7	4	5.5	3	2.5
8	2.5	7.5	2.5	2.8	.6	2.2	5	10	8	10	9	9	8	8	8	12	12	10	7	4	6	3	1.5	2.5
9	2	3	3	5	4	3	3	3.5	6	8	8	8	9.5	10	10	11	11	9	8	4.5	2.5	3.5	6.5	8.0
Average	4.5	4.8	2.8	4.6	2.5	2.7	4.2	6.7	7.3	8.7	8.2	8.7	8.7	9.0	8.7	10.7	10.3	9.0	7.7	5.2	4.2	4.0	3.7	4.3

TABLE XXXIV.—*Evaporation rate (in grams per hour) during Amaranthus retroflexus transpiration period at Akron, Colo., from July 7 to 9, 1914*

Date.	Hour ending—																							
	A. M.											Noon.	P. M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
July 7	0	0	0	0	20	40	60	260	460	580	680	800	840	840	760	640	440	368	220	130	60	60	20	0
8	0	20	20	0	0	0	220	440	460	760	840	920	1,000	880	840	760	640	540	300	180	50	40	40	40
9	40	40	40	20	20	50	110	280	600	740	860	940	980	980	860	800	650	500	270	150	80	60	80	90
Average	13	20	20	7	13	30	130	326	506	694	793	887	940	900	820	733	577	470	264	154	64	54	46	44
Percentage of maximum	1	2	2	1	1	3	14	35	54	74	85	94	100	96	87	78	62	50	28	16	7	6	5	5

The transpiration measurements (see Table XXIX) include six day records on three successive days in July. The corresponding physical measurements are given in Tables XXX to XXXIV, inclusive, and the hourly means are plotted in figure 8.

While these measurements were made during what we have termed "clear days," the sky was not wholly free from cumulus cloud during the period, and this is reflected in the radiation curve, which does not quite reach its normal value during the late morning hours.

Comparison with the conditions prevailing during the rye transpiration period, which extended over the two preceding weeks, will show that the evaporation was distinctly higher during the amaranthus period. The temperature during the latter period was slightly lower, but the saturation deficit was greater. Yet the transpiration graph of *Amaranthus retroflexus* gives no indication of the flattening which is so marked in the transpiration graph of rye. There appears then to be a marked difference in this respect in the response of the two plants to the march of radiation and other cyclic factors.

GENERAL DISCUSSION

It seems desirable at this point to summarize briefly the prevailing climatic conditions at Akron during the growth period of plants and more particularly during the transpiration periods included in the above determinations (Table XXXV). Akron is located in the rolling short-grass plains of northeastern Colorado. Absolutely clear days seldom occur, but often there are days with only a few light cumulus clouds in the sky, and during such days the plants are rarely shaded from the direct rays of the sun. Such brief interruptions in the direct radiation appear to have little influence on the hourly transpiration rate. On the other hand, there are many days during which cloudiness develops, especially in the afternoon, not infrequently accompanied by light rain and high wind. The number of days which may be classified as clear in the above-defined sense forms consequently a relatively small part of the growth period of the plants. The measurements presented in this paper have been made on practically cloudless days. The radiation intensity at midday on clear days in midsummer is normally about 1.4 calories per square centimeter per minute on a surface normal to the sun's rays. In the 1912 experiments the hazy condition of the atmosphere, together with the shading effect of the hail screen, combined to reduce the maximum radiation to 0.8 calorie during the wheat transpiration period, 1.02 calories during the oat transpiration period, and 1.05 calories during the sorghum measurements. The plants during the 1912 measurements were consequently obliged to dissipate only from 60 to 75 per cent as much solar energy as in the 1914 experiments.

TABLE XXXV.—*Summary of plant and environmental data*

CROP OF 1912

	Wheat.			
	Turkey.	Kharkov.	Kubanka.	Mean for all varieties.
Transpiration period	June 25 to July 11	June 20 to July 5	June 25 to July 8	June 20 to July 11
Date of cropping	Aug. 1	Aug. 1	Sept. 3	.....
Yield of dry matter.....gm.	344	366	270	327
Mean maximum transpiration.....gm. per hour	258	302	174	238
Maximum transpiration.....gm. per hour	320	450	260	.....
Mean maximum radiation, calories per sq. cm. per minute	.....	.....	.....	0.8
Mean maximum air temperature.....° F.	.....	.....	.....	86.1
Range in mean wind velocity	.....	.....	.....	1.6 to 7.5
Mean maximum transpiration per gram of dry matter harvested	0.75	0.83	0.65	0.73

CROP OF 1912

	Oats.	Sorghum.			
	Swedish select.	Minnesota Amber.	Milo.	Dwarf Milo.	Mean for all varieties.
Transpiration period	Aug. 4 to 18	Aug. 23 to 29	Aug. 25 to 29	Aug. 24 to 29	Aug. 23 to 29
Date of cropping	Aug. 23	Sept. 26	Sept. 27	Sept. 27	.....
Yield of dry matter.....gm.	411	667	509	434	537
Mean maximum transpiration, gm. per hour	271	412	430	354	408
Maximum transpiration....gm. per hour	464	.....	.....	.....	.....
Mean maximum radiation, calories per sq. cm. per minute	1.02	.....	.....	.....	1.05
Mean maximum wet-bulb depression	17.1	.....	.....	.....	26.7
Mean maximum saturation deficit, inches	0.602	.....	.....	.....	1.138
Mean maximum air temperature.....°F.	79.0	.....	.....	.....	91.7
Range in mean wind velocity	2.5 to 6.7	.....	.....	.....	2.4 to 8.7
Mean maximum transpiration per gram of dry matter harvested	0.66	0.62	0.84	0.81	0.76

CROP OF 1914

	Rye.	Alfalfa.			Amaranthus.
		Early period.	Whole period.	Late period.	
Transpiration period	June 22 to July 3	June 18 to 21	.....	Oct. 16 to 20	July 7 to 9
Date of cropping	July 25	July 11	.....	Oct. 26	July 14
Yield of dry matter.....gm.	186	157	.....	176	122
Mean maximum transpiration, gm. per hour	294	414	482	488	234
Mean maximum evaporation, gm. per hour	774	867	710	616	940
Mean maximum radiation, calories per sq. cm. per minute	1.38	1.34	1.28	1.21	1.38
Mean maximum wet-bulb depression	22.8	25.7	25.0	26	27.3
Mean maximum saturation deficit, inches	0.820	1.043	0.827	0.811	1.056
Mean maximum air temperature.....° F.	82.6	89	79.7	77	88.5
Range in mean wind velocity	3.9 to 8.7	1.5 to 7	3.5 to 8.3	3.8 to 14.4	2.5 to 10.7
Mean maximum transpiration per gram of dry matter harvested	1.58	2.64	.....	2.77	1.92

Since transpiration and evaporation are similarly affected by environmental factors, the loss of water from a free-water surface affords a good summation of the intensity of such factors. The total evaporation from a tank 8 feet in diameter with the water surface at ground level at Akron during the months from April to September, inclusive, is 44 inches, based on the records for seven seasons, compared with 33 inches at Dickinson in western North Dakota, 53 inches at Amarillo in the Panhandle of Texas, and 57 inches at Yuma, Ariz. In general, the evaporation increases as one proceeds from north to south through the Great Plains, and the same condition, though less marked, prevails from east to west. The transpiration conditions at Akron are probably as severe as may be found in cultivated areas east of the Rockies in this latitude (40° N.) or to the north of this parallel.

Hourly evaporation measurements with the shallow, blackened tank were not made in 1912. The evaporation rate in 1914 was highest during the amaranthus period, as would be expected from a consideration of the intensity of the environmental factors. The mean maximum evaporation rate for the different periods during the hours near midday ranged from 700 to 900 gm. per hour from a tank of 6,540 sq. cm. in area.[1]

The highest temperatures and the greatest saturation deficits were encountered during the sorghum and amaranthus transpiration periods; yet these conditions produced no flattening of the peak of the transpiration curve of either plant, which is so marked in the case of wheat and rye. The lowest mean temperature and the smallest saturation deficit

[1] A loss of 1,000 gm. from the small tank corresponds to a loss of 0.0386 inch from the 8-foot tank referred to above, based on continuous records for the period, June 16 to September 19, 1914. The large tank loses more slowly during the forenoon, but more rapidly during the night. This is due to the heat capacity of the large tank. The records based on 24-hour periods show good agreement between the two tanks. To those who are more familiar with evaporation as measured by Livingston's atmometer, the following comparison with the shallow blackened evaporation tank used in our experiments will be of interest. The hourly evaporation graph of the porous-cup atmometers does not agree in form with the evaporation graph from the tank. The atmometers show a marked lag during the middle of the day as compared with the evaporation taking place from the tank. This might be anticipated, since the tank receives only the vertical component of the radiation, while the candle type of atmometer receives a smaller percentage of the total radiation at midday in midsummer than earlier or later in the day, due to the vertical walls. The difference is, however, very pronounced even with the new spherical form of porous cup. It is consequently impossible to establish a definite ratio between the evaporation from the Livingston atmometers and the shallow tank used in our experiments. The average ratio may, however, be given. From 6 a. m. to 6 p. m., on August 13 and 14, 1915, an evaporation of 1,000 c. c. from the tank corresponded to an evaporation of 6.5 c. c. from the white candle-type atmometers (1913); of 7.5 c. c. from the same type (1915); of 8.3 c. c. from the white, spherical type (1915); and of 10.9 c. c. from the black candle type (1915). The loss from the atmometers corresponding to 1,000 gm. loss from the shallow tank for different parts of the day is as follows:

Type of atmometer.	6 to 10 a. m.	10 a. m. to 2 p. m.	2 to 6 p. m.
White candle type (1913)	7.2	5.1	8.6
White candle type (1915)	8.2	5.8	10.0
White spherical type (1915)	9.1	6.7	10.3
Black candle type (1915)	14.0	8.4	12.9

During the night the atmometers each lost about 3 gm. of water, while the tank showed a slight gain due to deposition of dew. None of these atmometers had ever been used in other measurements, and distilled water was used in all cases. The values given are based on the means of determinations with four atmometers of each type, after the observed evaporation from each atmometer had been multiplied by the standardization coefficient supplied with the apparatus.

occurred during the oat transpiration period. This may account for the fact that the flattening of the transpiration curve of oats is not so marked as in the case of the other cereals.

The wind velocity during these experiments was higher during the daytime than during the night hours. There is a fairly well-defined maximum between 7 and 10 o'clock and another secondary maximum in the afternoon. Wind-still periods seldom occurred.

In Table XXXV are summarized the mean maximum values of the transpiration, evaporation, radiation, saturation deficit, and temperature for each period; and the yield, time of harvest, and the period during which transpiration measurements were made. The range in mean wind velocity and the mean maximum transpiration per gram of dry matter harvested have also been added to the table.

A comparison of the data for the three varieties of wheat shows a close agreement. Kharkov produced the highest yield and transpired at the highest rate. Kubanka produced the least dry matter and transpired at the lowest rate. On the basis of dry matter produced Kharkov transpired most rapidly and Kubanka least rapidly. From a consideration of unpublished data on the transpiration of cereals from seed time to harvest, these observations appear to have been taken during the period of maximum transpiration for the crops considered.

On the basis of transpiration throughout the total period of growth, the relative transpiration of Kharkov and Türkey wheat was the same—i. e., 365 ± 6 and 364 ± 6 gm. of water, respectively, for each gram of dry matter produced. Kubanka transpired relatively more—i. e., 394 ± 7 gm. of water for each gram of dry matter.

Oats transpired somewhat less rapidly than wheat in proportion to the amount of dry matter produced. A consideration of the temperature data shows the mean maximum temperature for the oat period to be about 7 degrees lower than for the wheat period. This difference in temperature and the resulting difference in humidity would be sufficient to account for the lower rate of transpiration of oats compared with wheat. On the basis of total transpiration, oats consumed 423 ± 5 gm. of water for each gram of dry matter produced, or 7 per cent more than Kubanka wheat.

Three different varieties of sorghum were used in the transpiration measurements—Minnesota Amber, milo, and Dwarf milo. The plants were apparently at the height of their transpiration during the measurements. The mean maximum transpiration rate of sorghum was higher in proportion to the dry matter harvested than for oats or wheat, but the physical conditions favored a more rapid transpiration during the sorghum period, as is shown by a comparison of the temperature, radiation, and saturation-deficit data. The slope of the sorghum transpiration curve near the peak is also much greater than for either wheat or oats.

The transpiration during the whole period of growth of sorghum, when based on dry matter produced, is practically the same for the three varieties here considered. Minnesota Amber transpired 239±2 gm. of water for each gram of dry matter produced; Dwarf milo, 273±4 gm.; and milo, 249±3 grams.

The transpiration rate of rye, when based on the dry matter harvested, is much higher than for any other crop included in the 1912 water-requirement measurements. This is due in part to the more extreme atmospheric conditions prevailing during this period and in part to the higher water requirement of rye, which is 39 per cent higher than Kubanka wheat and 15 per cent higher than Swedish Select oats.

The data presented during the long period for alfalfa were based on plants which yielded different amounts of dry matter. In order to make the comparison more exact, two short periods have been presented. The environmental conditions were somewhat more extreme during the early period, as is shown by a comparison of radiation, temperature, and saturation deficit. The evaporation rate was also higher. On the basis of dry matter harvested, the transpiration during the two periods was the same. It is necessary in this connection to consider the size of the plant at the actual time of the measurements. The late-period crop was harvested 6 days after the period when the transpiration measurements were made, while the early-period crop was harvested 20 days after the termination of the transpiration measurements. It is evident, therefore, that the ratio of transpiration rate to dry matter of the early-period crop would have been considerably higher had this crop been harvested soon after the transpiration measurements were completed.

The most severe environmental conditions in 1914 were encountered during the amaranthus period. Solar radiation was greater and saturation deficit, air temperature, and evaporation higher. On the basis of dry matter, amaranthus transpired less than alfalfa, but more than rye. On the basis of the whole period of growth, the water requirement of amaranthus was much less than rye, the higher rate of transpiration shown in the data here presented being due to the unusually severe conditions prevailing during this period.

While the writers are considering the data in this paper primarily from the standpoint of the relative transpiration rate of the different plants and are not particularly concerned with absolute values, it is interesting to find that the data here presented conform as nearly as can be expected to the relative transpiration rates of the different plants as determined from the water-requirement measurements.

COMPARISON OF THE FORM OF THE CURVES

In order that a more accurate comparison may be made between the form of the transpiration graph and that of the several environmental factors, the mean hourly values presented in the preceding tables have

also been expressed in terms of percentage of the maximum. In the case of temperature and wet-bulb depression, the calculation has been based on the maximum range—i. e., the mean minimum is taken as zero on the scale. The data for the various crops reduced to this uniform basis are presented in figures 9 to 15, inclusive, the axis of abscissas representing time and the axis of ordinates the percentage of the mean daily maximum (or mean daily range).

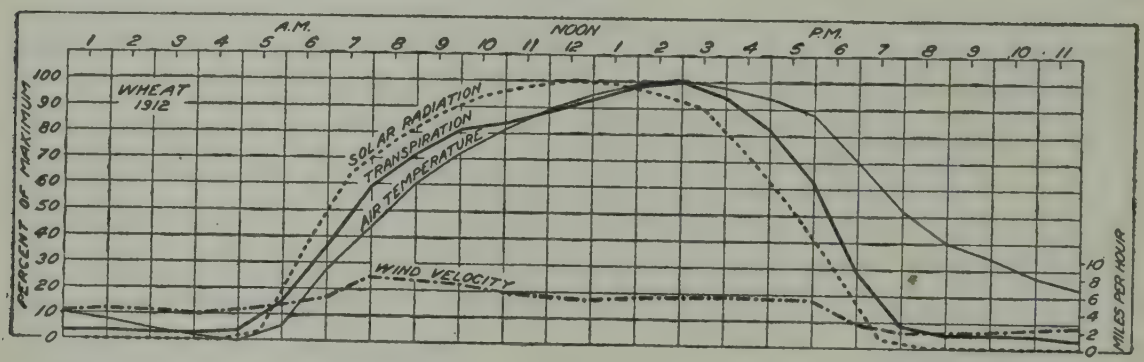

FIG. 9.—Graphs showing transpiration of wheat and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

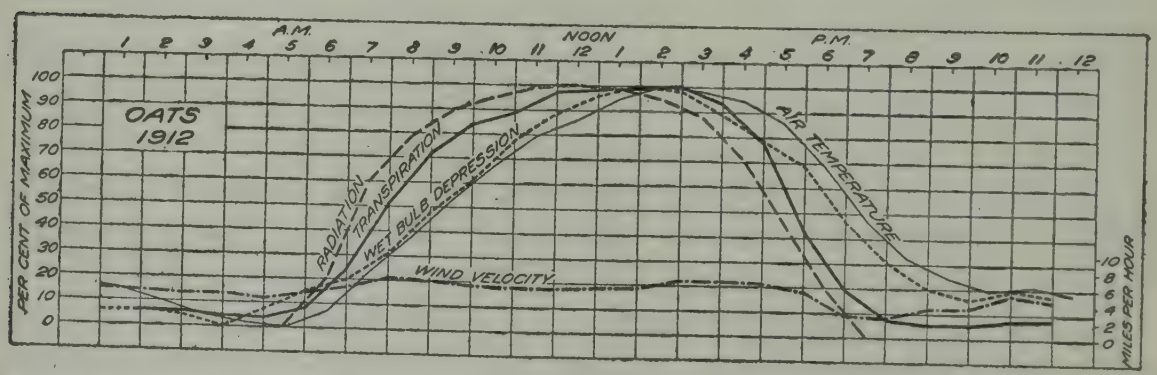

FIG. 10.—Graphs showing the hourly transpiration of oats and the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

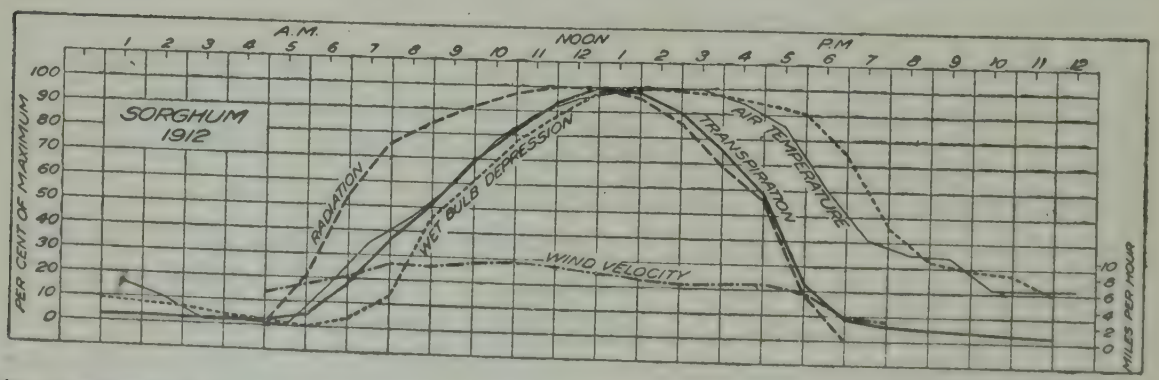

FIG. 11.—Graphs showing the hourly transpiration of sorghum and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

An inspection of the charts will show that the radiation graph rises in advance of the other cyclic environmental factors. This is to be expected, since the change in radiation is the primary cause of the cyclic change of the other components. For the same reason the radiation also rises in advance of the transpiration and falls either in advance of it, as in

the case of the three cereals wheat, oats, and rye, or approximately with the transpiration, as in the case of sorghum, alfalfa, and amaranthus.

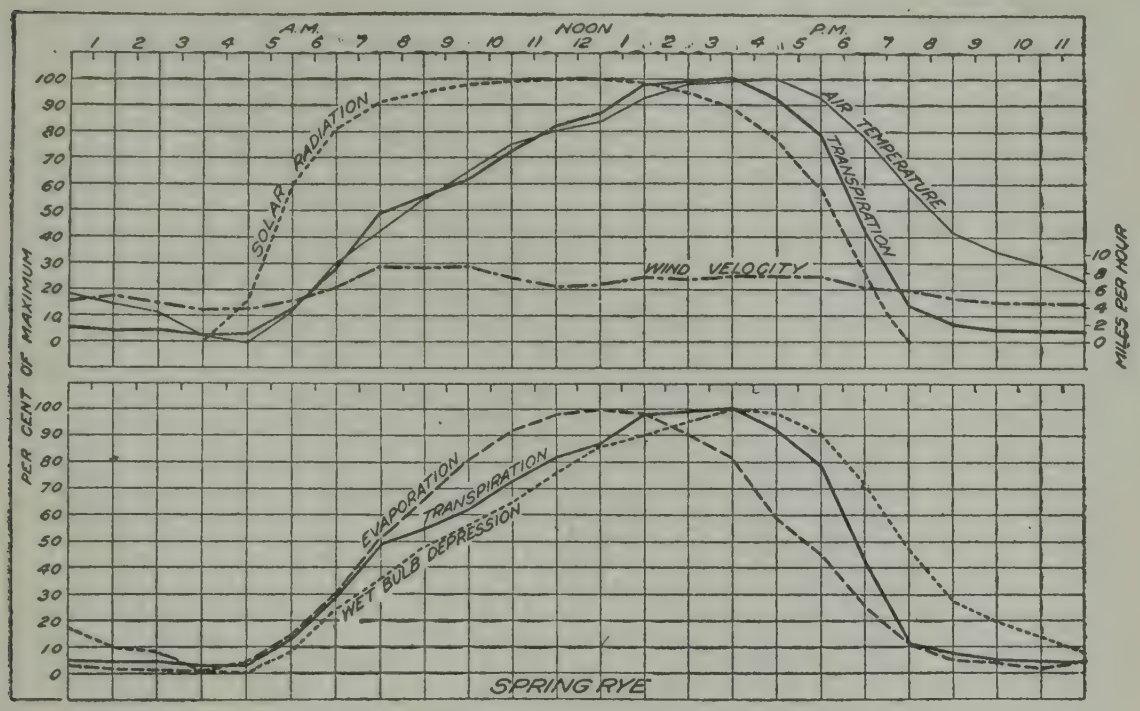

FIG. 12.—Graphs showing hourly transpiration of spring rye and the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

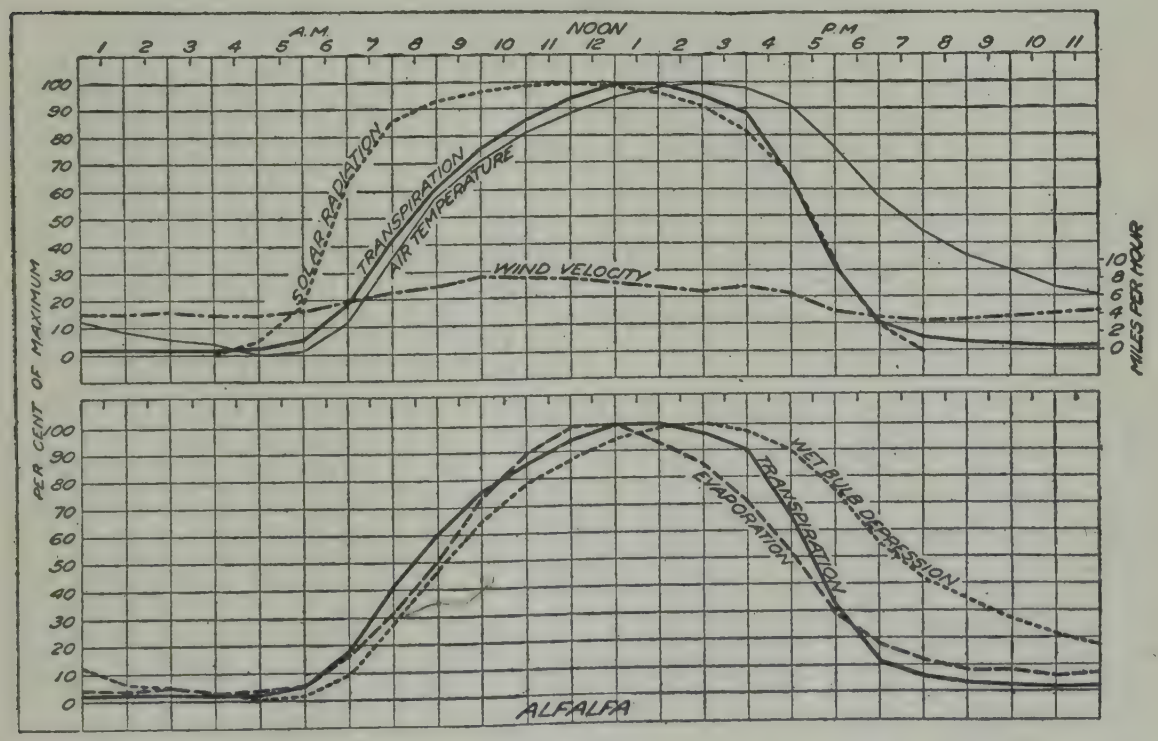

FIG. 13.—Graphs showing the hourly transpiration of alfalfa and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

This is clearly shown in figure 16, in which the two graphs are plotted for each plant.

The transpiration rises in advance of the temperature in the case of wheat, oats, and alfalfa; approximately with the temperature for rye

and sorghum; and later than the temperature for amaranthus. This is in evidence in figure 17, in which these two graphs alone are plotted for each plant measured. The transpiration in the afternoon always falls off far more rapidly than the temperature, and when the transpiration has reached the night level the temperature is still above the minimum by an amount corresponding roughly to one-third the daily range.

The wet-bulb depression and the air-temperature curves are very similar in form, owing to the fact that with a uniform absolute-moisture content of the air the former curve is determined strictly by the latter.

The transpiration rises in advance of the wet-bulb depression (fig. 18) in every instance except amaranthus, in which the graph starts later but crosses the wet-bulb depression curve about 9 a. m. The transpiration falls more rapidly than the wet-bulb depression in every instance.

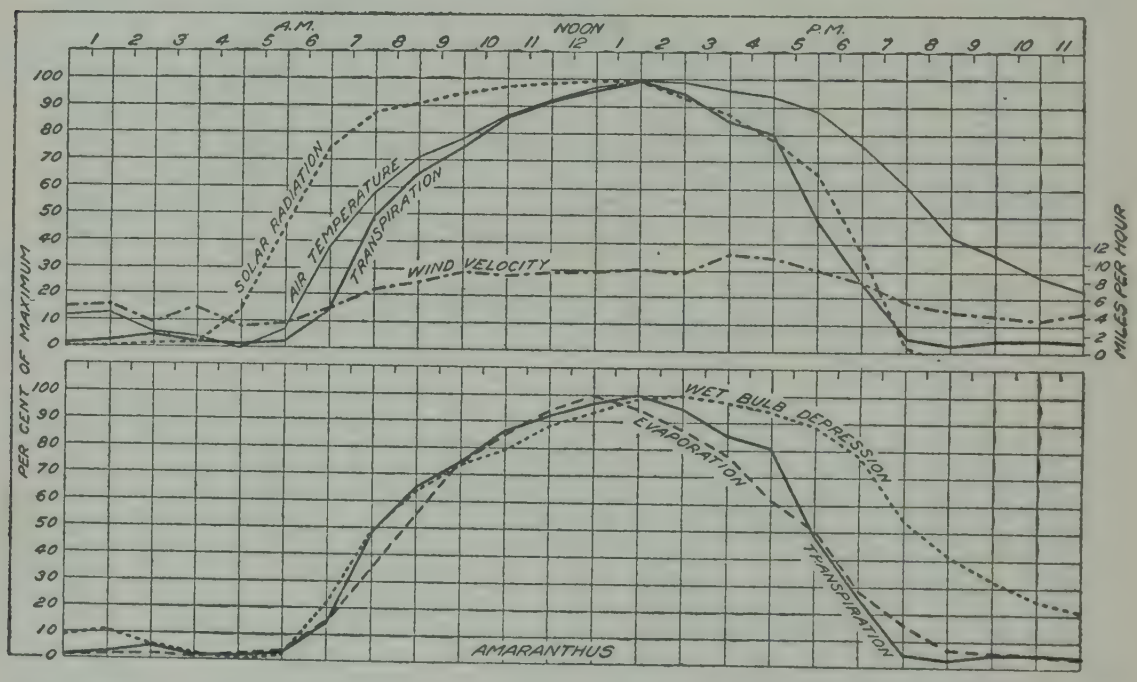

FIG. 14.—Graphs showing the hourly transpiration of *Amaranthus retroflexus* and the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

The evaporation rises later than the transpiration graph (fig. 19) in the case of alfalfa and amaranthus, owing to the fact that the tank evaporation is determined largely by the vertical component of the radiation, while isolated pots of plants probably receive radiation in excess of the vertical component. In the case of rye, the two graphs coincide during the early morning hours, but a marked depression of the transpiration curve from the evaporation graph occurs at 8 a. m., this difference persisting until after the evaporation graph has passed its maximum. The comparison of the two graphs brings out very strikingly the depression in the transpiration graph of rye during the morning hours, to which attention has already been called and which is a common feature of the cereals so far investigated.

The evaporation graph in the early afternoon falls in advance of the transpiration graphs, but owing to the greater slope of the transpiration

graphs in the late afternoon the two curves tend to reach the night level at about the same time.

DISTRIBUTION OF TRANSPIRATION IN RELATION TO SOLAR RADIATION

In Table XXXVI is given a summary of the data represented by the radiation and transpiration graphs shown in figure 16. The second column of this table shows the relative radiation received by the different

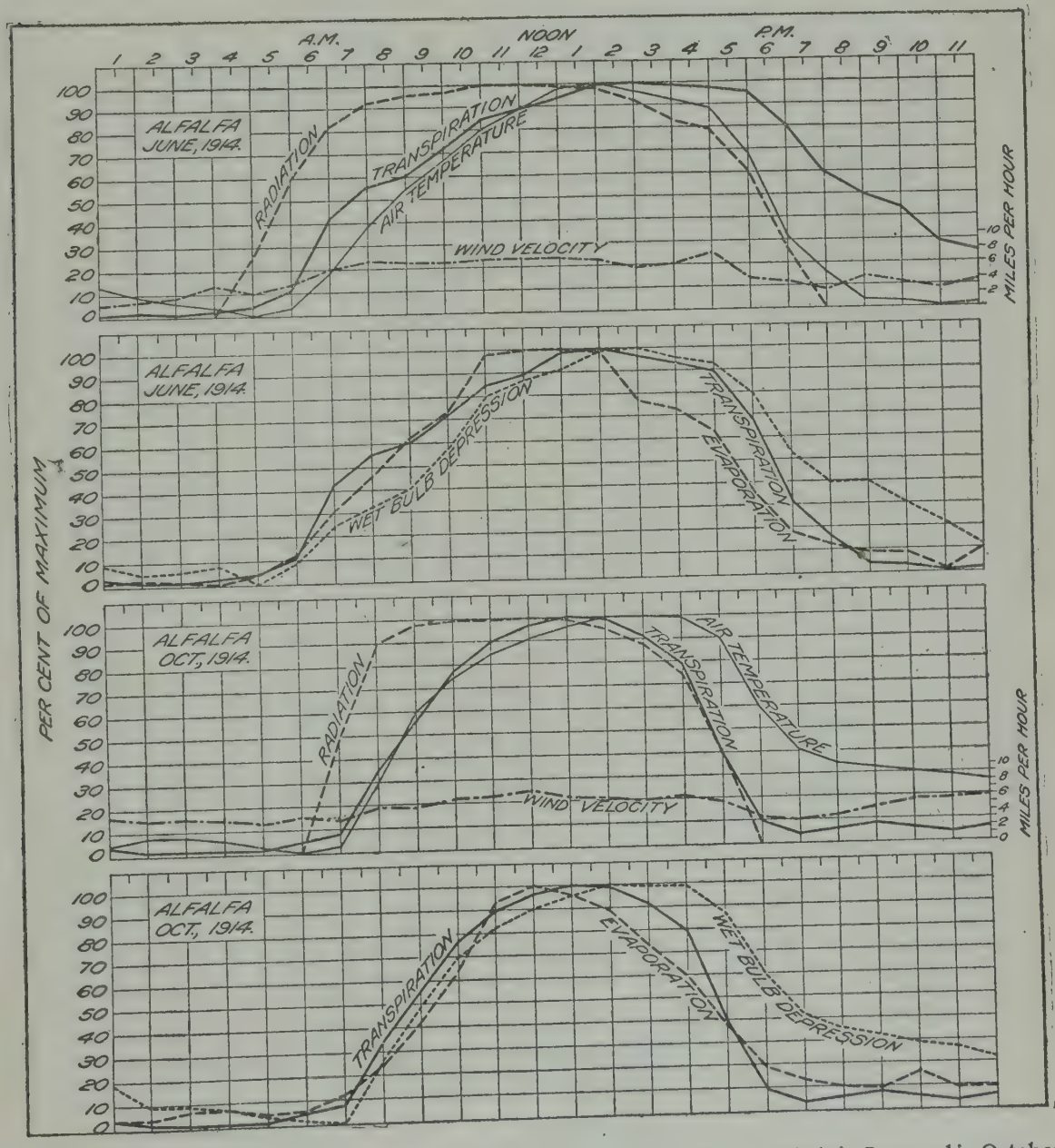

FIG. 15.—Graphs showing the hourly transpiration values of alfalfa for short periods in June and in October, with the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

crops, giving in arbitrary units the integrated area bounded by the radiation curve and the time axis. The integrated transpiration obtained in a similar manner is given in the third column. In the fourth column is given the ratio of the integrated transpiration to the integrated radiation for each particular crop.

It will be seen from these figures that the integrated transpiration for wheat and oats slightly exceeds the integrated radiation and that the reverse is true for rye, sorghum, amaranthus, and alfalfa. The transpiration curves for sorghum, amaranthus, and alfalfa lie almost wholly within the radiation curve. The ratio of the transpiration area to the radiation area is also low in the case of spring rye, owing to the comparatively low rate of transpiration during the morning hours.

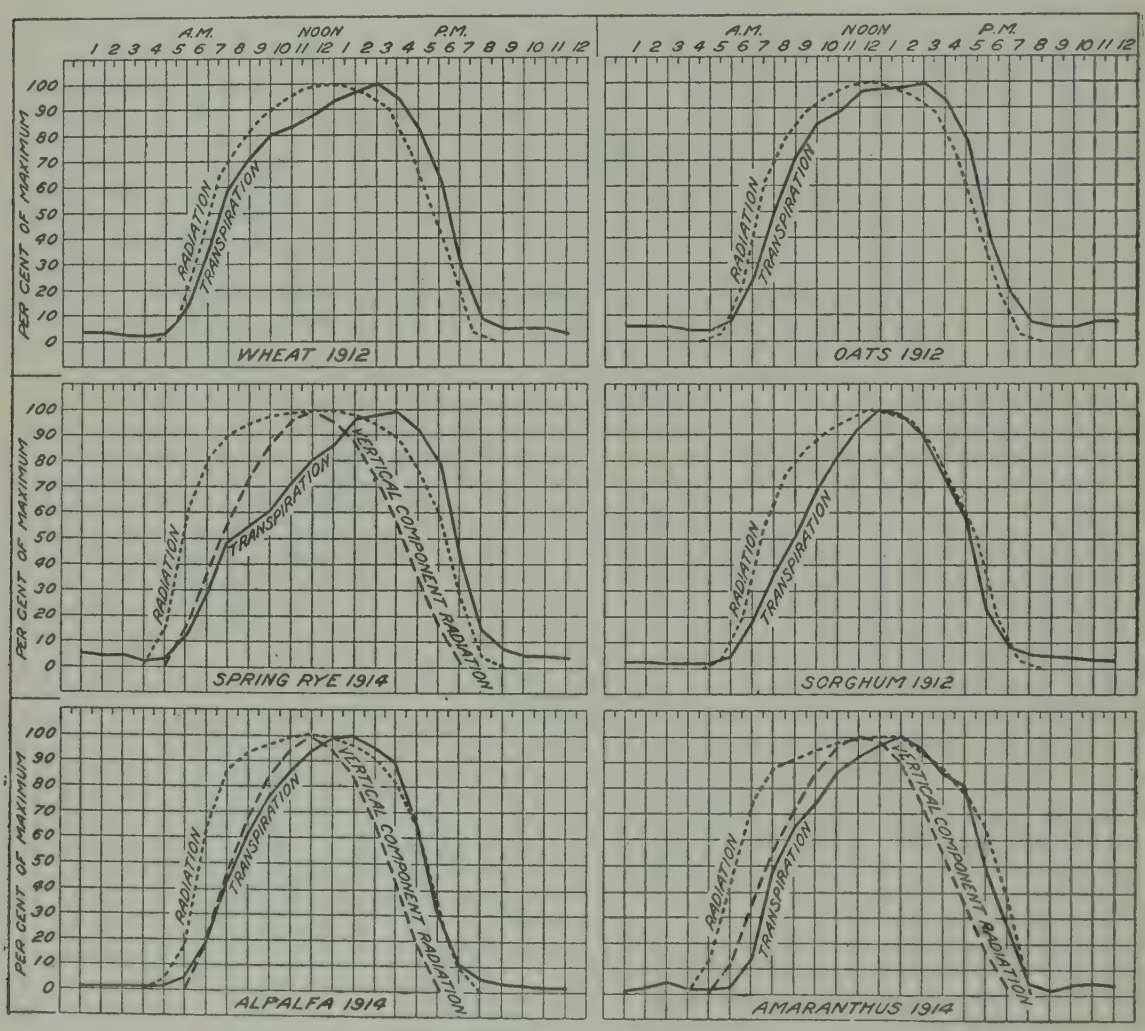
A.M.
NOON
P.M.
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
PER CENT OF MAXIMUM
100 90 80 70 60 50 40 30 20 10 0
RADIATION
TRANSPIRATION
VERTICAL COMPONENT RADIATION
WHEAT 1912
OATS 1912
SPRING RYE 1914
SORGHUM 1912
ALFALFA 1914
AMARANTHUS 1914

FIG. 16.—Comparison of the form of transpiration graphs with the graphs representing the total radiation and the vertical component of the radiation.

TABLE XXXVI.—*A comparison of radiation and transpiration based on the area inclosed by the graphs in figure 17*

Plant.	Area bounded by—		Ratio of transpiration to radiation area.	Transpiration.					
	Radiation graph.	Transpiration graph.		Area for daylight hours.	Daylight.	Night.	A. M.	P. M.	11 a. m. to 3 p. m.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Wheat	302	310	1.03	298	96	4	44	56	37
Oats	289	303	1.05	286	94	6	44	56	39
Rye	357	306	.86	290	95	5	38	62	36
Sorghum	283	253	.89	240	95	5	43	57	45
Amaranthus	346	284	.82	275	97	3	42	58	40
Alfalfa	315	271	.86	264	97	3	44	56	43

The last portion of Table XXXVI gives the relative transpiration for different parts of the day. The percentage of the transpiration taking place during daylight is very uniform, ranging from 94 per cent for oats to 97 per cent for amaranthus and alfalfa. The transpiration during the night is remarkably low, ranging from 3 per cent for amaranthus and alfalfa to 6 per cent for oats. The data as presented represent the integration of the transpiration and radiation for hourly intervals, so that the transpiration for the hour interval during which sunrise (or sunset) occurred has been included as daylight transpiration. The ratio can be more accurately determined from the automatic records, which show an

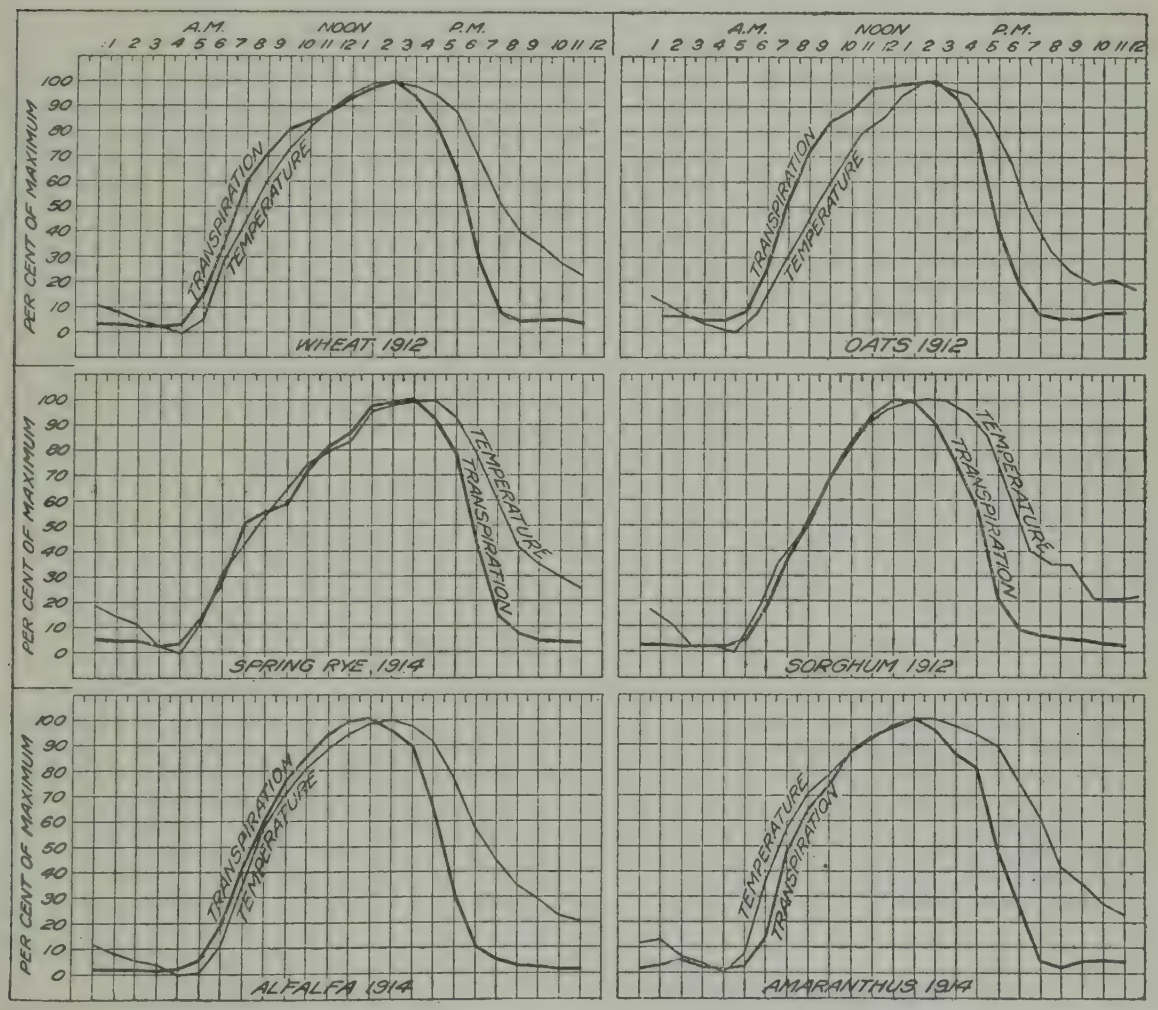

FIG. 17.—Comparison of the transpiration graphs plotted in percentage of the maximum with the temperature graphs plotted in percentage of the maximum range.

average night transpiration less than 5 per cent of that occurring during daylight. This low night transpiration is significant when we consider that the temperature and the saturation deficit are relatively high during the early hours of the night and that the dew point is seldom reached at Akron. The wind velocity at night is also at least one-half the average daylight velocity.

It will be seen from Table XXXVI that the transpiration in the forenoon is lower than in the afternoon, the difference being greatest in the case of rye and least in the case of wheat, oats, and alfalfa. For the

group of plants as a whole, 43 per cent of the transpiration took place before noon and 57 per cent in the afternoon, while the average radiation during the period was slightly greater in the forenoon.

In the last column of the table is given the percentage of transpiration taking place between 11 a. m. and 3 p. m. While these figures are not directly comparable, owing to the difference in the length of the day—i. e., in the number of daylight hours— it is clear that from one-third to

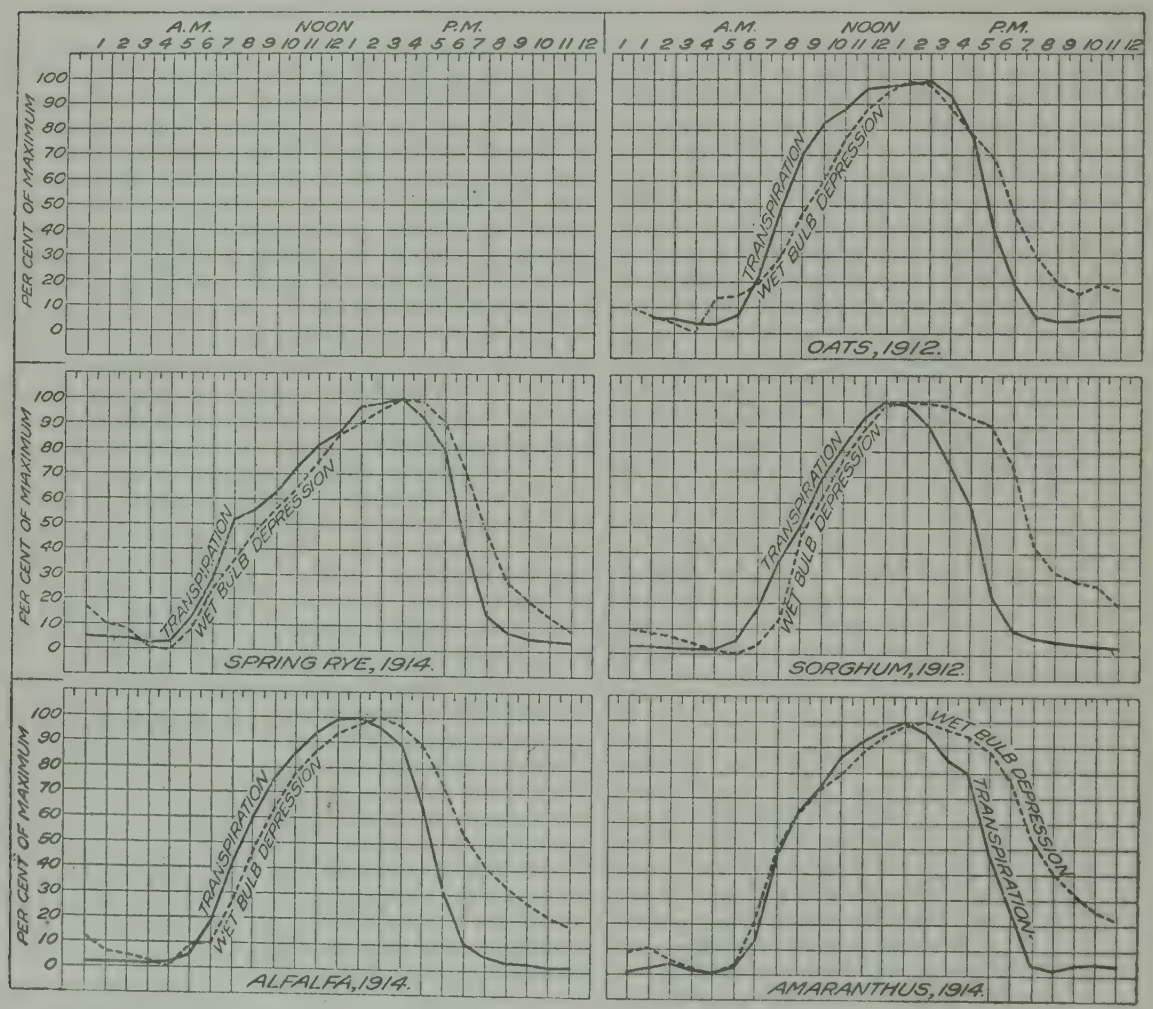

FIG. 18.—Comparison of transpiration with wet-bulb depression, both plotted in percentage of the maximum range.

one-half of the transpiration during the 24-hour period takes place from 11 a. m. to 3 p. m.

RATIO OF TRANSPIRATION TO EVAPORATION

Transpiration is often regarded as evaporation modified to some extent by plant structures and plant functions. Both are influenced by radiation, temperature, saturation deficit, and wind. Because of the similarity of the two processes, the evaporation rate has often been used as a standard to which the transpiration is referred.

Livingston (1906 and 1913) has given special attention to the relation of transpiration to evaporation, and has applied the terms "relative

transpiration," "transpiring power" (Livingston and Hawkins, 1915), to the ratio of the transpiration rate to the evaporation rate of his porous-cup atmometer. It has been shown[1] that the graphs representing transpiration and the evaporation from the porous-cup atmometer are similar in form, but that their maxima do not as a rule occur at the same time in plants exposed to extreme conditions. Furthermore, when the

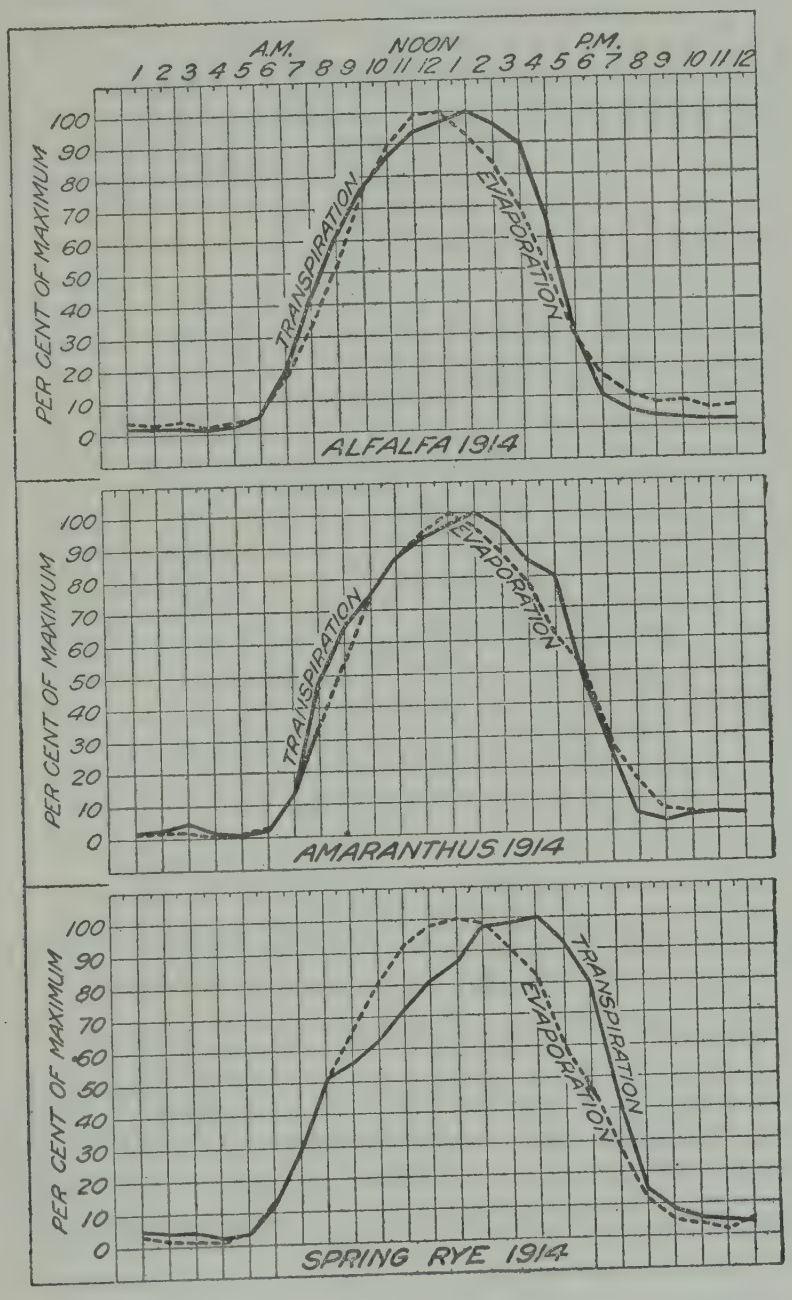
A.M.
NOON
P.M.
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
PER CENT OF MAXIMUM
100 90 80 70 60 50 40 30 20 10 0
TRANSPIRATION
EVAPORATION
ALFALFA 1914
AMARANTHUS 1914
SPRING RYE 1914

FIG. 19.—Comparison of the transpiration with the evaporation from a free-water surface in a shallow, blackened tank, both plotted in percentage of the maximum range.

ratio of the transpiration to evaporation (the relative transpiration) is plotted against time, the daily graph usually shows two maxima, one in the morning and a second in the afternoon.

Graphs representing the ratio of the transpiration rate of rye, alfalfa, and amaranthus to the evaporation rate are given in figure 20 and show

[1] See also Shreve, 1914; Bakke, 1914.

the maxima referred to in the investigations cited. One maximum occurs in the morning about 7 or 8 o'clock, and a second and greater maximum is found in the afternoon between 4 and 6 p. m.[1] In other words, the transpiration graph shows a tendency to rise earlier in the morning and fall later in the afternoon than the evaporation graph. This is evident in each of the three graphs presented in figure 20.

This result is capable of two quite dissimilar interpretations. If the assumption is made that evaporation constitutes a correct summation of the influence of environment on transpiration, it follows logically that the departure of the transpiration-evaporation ratio from a constant value is due to a decrease or increase in the transpiration coefficient. It must,

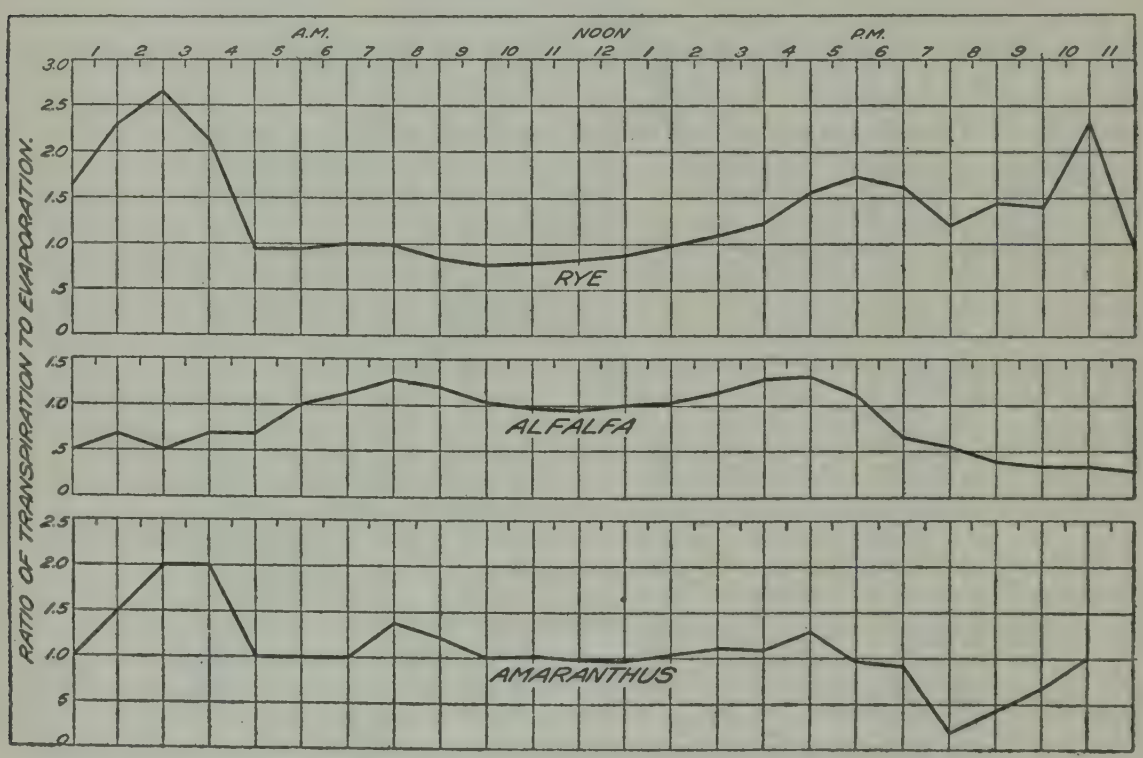

FIG. 20.—Graphs showing hourly ratio of transpiration to evaporation as plotted in figure 19.

however, be recognized that all evaporimeters do not respond to their environment in the same way. A large deep tank does not have the same daily graph as a shallow tank. A filter-paper evaporimeter does not follow the graph of the porous-cup atmometer. If none of these agree, can it be said without further proof that the evaporation rate of any one of them is proportional to the transpiration rate of a plant which responds *freely* to its environment? The fact that the transpiration graph is so uniformly asymmetrical with respect to noon in our determinations and that the evaporation graph is so uniformly symmetrical would indicate that the two processes were not controlled in the same way by the physical factors of the environment. The writers are inclined to the belief that

[1] The hourly values of transpiration and evaporation at night are so small that the observational errors make the ratio uncertain, and the night ratios will consequently not be considered at this time.

the departure of the transpiration from evaporation should not be taken as proof of a change in the transpiration coefficient of the plant and that it is safer for the present not to base conclusions on this assumption but instead to consider directly the factors which influence both transpiration and evaporation.

CORRELATION BETWEEN TRANSPIRATION AND ENVIRONMENTAL FACTORS

Two methods have been employed by the writers in making a quantitative investigation of the relationships existing between the transpiration of the plant and the intensity of its environment: (1) The coefficient of correlation between the transpiration and a given environmental factor has been computed as a basis for the determination of the relative influence of the various environmental factors and (2) the relationship between the mean hourly transpiration and the hourly values of the several environmental factors has been computed by the method of least squares, and the relative weights of the different environmental factors determined from the coefficients of the resulting equation. Such a reduction of the data appears highly desirable, for it affords a means of comparison independent of the personal element. The results of the correlation reductions will first be considered.

In computing the correlation coefficients,[1] the individual hourly observations as presented in Tables I to XXVI were used. The data in each instance embrace not less than three days' observations with the transpiration measurements in duplicate, so that the number of pairs of terms—i. e., the "population" considered—approximated 144 for the 3-day periods in the transpiration correlations and in other cases exceeded this number.

The correlation coefficients of the transpiration rate of alfalfa, amaranthus, and rye, with the intensity of the several environmental factors, are presented in Table XXXVII, together with the probable error of the correlation coefficient in each case.

[1] For a presentation of the theory and the method of computing correlation coefficients, see Yule (1912) and Davenport (1907).

TABLE XXXVII.—*Correlation between transpiration and environmental factors*

Plant, period, and components.	Correlation coefficient.	Plant, period, and components.	Correlation coefficient.
Alfalfa (long period, Sept. 10 to Oct. 20, 1914):		Alfalfa (June 18, 19, 21, 1914)—Continued.	
Radiation and transpiration	0.840±0.009	Wind velocity and transpiration	0.626±0.036
Temperature and transpiration	.819± .011	Wind velocity and radiation	.641± .046
Wet-bulb and transpiration	.822± .011	Vertical radiation and transpiration	.818± .013
Evaporation and transpiration	.838± .011	Amaranthus (July 7 to 9, 1914):	
Wind velocity and transpiration	.485± .026	Radiation and transpiration	.844± .016
Wind velocity and radiation	.302± .030	Temperature and transpiration	.849± .016
Alfalfa (Oct. 16 to 20, 1914):		Wet-bulb and transpiration	.842± .016
Radiation and transpiration	.886± .010	Evaporation and transpiration	.946± .006
Temperature and transpiration	.859± .012	Wind velocity and transpiration	.683± .031
Wet-bulb and transpiration	.843± .013	Wind velocity and radiation	.776± .032
Evaporation and transpiration	.929± .006	Vertical radiation and transpiration	.863± .014
Wind velocity and transpiration	.353± .039	Rye (June 22 to July 3, 1914):	
Wind velocity and radiation	.275± .084	Radiation and transpiration	.820± .014
Vertical radiation and transpiration	.862± .011	Temperature and transpiration	.854± .011
Alfalfa (June 18, 19, 21, 1914):		Wet-bulb and transpiration	.748± .018
Radiation and transpiration	.861± .015	Evaporation and transpiration	.894± .033
Temperature and transpiration	.788± .021	Wind velocity and transpiration	.376± .036
Wet-bulb and transpiration	.852± .008	Wind velocity and radiation	.353± .047
Evaporation and transpiration	.888± .012	Vertical radiation and transpiration	.766± .017

An inspection of the correlation table will show that, excluding evaporation, the highest correlation is obtained between radiation and transpiration. The correlation coefficient for these components is remarkably uniform for the different crops and periods, ranging from 0.82 to 0.88, the lowest value occurring in the case of rye, as one might expect from the form of the transpiration graph.

The similarity in the form of the composite graphs for air temperature and wet-bulb depression would lead to the expectation that their correlation coefficients with transpiration would be similar and the coefficients are in fact nearly the same. The only exceptions are (1) alfalfa (June period), in which the wet-bulb depression shows the higher correlation with transpiration; and (2) rye, in which temperature is the more closely

correlated. Reference to figures 12 and 17 shows the unusually close agreement between the composite transpiration graph for rye and the temperature graph.

The correlation coefficient of temperature (or wet-bulb depression) and transpiration also agrees approximately with that of radiation and transpiration. In other words, it appears from a consideration of these coefficients that radiation, temperature, and wet-bulb depression show an equally close association with the daily transpiration cycle. The correlation of temperature and wet-bulb depression with transpiration may, however, be looked upon as being in part associative with radiation rather than causative, as will appear from the following considerations.

The degree of correlation[1] between radiation and transpiration (from 0.82 to 0.88) indicates that the radiation determines the transpiration to the extent of from 0.67 to 0.77, the square of the correlation coefficients, if radiation is regarded as the primary causative factor. The remainder (0.33 to 0.23) is to be ascribed to other factors. If temperature is taken as a causative factor of transpiration, the correlation coefficients show a dependence of transpiration upon temperature of from 0.62 to 0.74; but this is far in excess of the remainder (0.33 to 0.23) to be accounted for. In other words, the sum of the squares of the two correlation coefficients is in excess of unity. This means, then, that temperature and radiation are intercorrelated. A similar intercorrelation exists between radiation and wet-bulb depression, and an exact differentiation is impossible. However, since these factors are physically dependent upon radiation, we may assign to radiation the total effect indicated by the correlation coefficient, keeping always clearly in mind the assumption involved. On this basis the radiation intensity determines two-thirds to three-fourths of the transpiration at Akron on clear days; or all other factors combined have only from one-third to one-half the influence of radiation.

On the other hand, if it is preferred to look upon radiation, temperature, and wet-bulb depression as direct independent causative factors (which must also be recognized as involving a specific assumption to this effect), then it is evident from Table XXXVII that these factors play approximately an equal part in determining transpiration on clear days. Not only are the correlation coefficients very nearly the same for the different factors with a given crop, but they vary but slightly for the different plants investigated.

[1] While a correlation coefficient of unity denotes perfect correlation, a correlation coefficient of less than unity must not be interpreted as determining the relationship in proportion to the magnitude of the correlation coefficient, for even in the case of a primary causative factor the relationship can not be greater than the square of the correlation coefficient. For example, a correlation coefficient of 0.707 between a causative and a resultant term indicates a dependence of the latter upon the former of 0.5—i. e., the square of 0.707. This may be easily demonstrated by computing the correlation coefficient between either of two series of numbers, each having a normal frequency distribution, with the product of one series by the other. The correlation coefficient of the product series with either primary series will be found to be 0.707. In other words, each series determines the product series to the extent of 0.5, while the two series together determine the product series absolutely.

In order to decide between these two assumptions, other evidence is necessary; and this may be found in a consideration of the transpiration during the night—i. e., when the radiation received by the plants is nil.

In Table XXXVIII are summarized the transpiration and wet-bulb depression (in percentage of the maximum) and the air temperature (in percentage of the maximum range) for the hours 3 to 4 a. m. and 8 to 9 p. m. It is evident from the table that a simultaneous diminution in the wet-bulb depression of one-fourth of its maximum and in temperature of one-third of its maximum range results in a drop of only 3 per cent in the transpiration rate. This would seem to indicate that the high correlation obtained between transpiration and air temperature (or wet-bulb depression) is largely due to the direct correlation between radiation and temperature (or wet-bulb depression).[1]

TABLE XXXVIII.—*Comparison of transpiration, temperature, and wet-bulb depression at 3 to 4 a. m. and 8 to 9 p. m.*

Crop or period.	Per cent of maximum transpiration.			Per cent of maximum temperature.			Per cent of maximum wet-bulb depression.		
	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.
Wheat	3	5	2	3	40	37	.....	.....	.....
Oats	4	6	2	2	24	22	21	37	16
Rye	3	7	4	3	42	39	17	39	22
Sorghum	2	5	3	2	34	32	25	48	23
Amaranthus	1	3	2	4	42	38	12	45	33
Alfalfa	2	3	1	4	35	31	15	42	27
June period	2	3	1	3	49	46	19	47	28
October period	1	7	6	5	32	27	2	38	36
Mean	.....	.....	3	.....	.....	34	.....	.....	26

If we ascribe to radiation a causative effect equal to that indicated by the correlation coefficient with transpiration, it becomes possible also to investigate the influence of wind velocity on transpiration by a process of elimination similar to that employed above.

Transpiration in still air is somewhat less than in moving air, since the latter tends to reduce the distance that the transpired moisture must move in order to find free-air conditions. In other words, the wind tends to increase the diffusion gradient, and so increases the transpiration (or evaporation) rate. But a slight movement appears to satisfy this condition, and the correlation coefficients between wind and transpiration (Table XXXVII) show that the variation in wind at Akron, where some wind nearly always occurs, has little influence on the trans-

[1] In opposition to this view it may be argued that the plants from 3 to 4 a. m. are more turgid than from 8 to 9 p. m. This is undoubtedly true; but it is also true that during the last named period the plants are more turgid than at 2 or 3 p. m., the period during which the maximum transpiration rate was observed.

piration rate. In arriving at this conclusion it is again necessary to consider the correlation not only between wind and transpiration, but also between wind and radiation. If the wind influences transpiration independently of its association with radiation, the wind velocity must show a higher correlation with transpiration than with radiation. This occurs only during the long alfalfa period, in which there appears to be a slight effect due to wind. In all other cases the wind correlation with transpiration differs from the wind correlation with radiation by an amount not greater than the probable error of the difference. Here, again, we are making the specific assumption that the radiation is the primary causative factor, so that if wind is associated with transpiration to an extent no greater than with radiation its effect on transpiration is slight. This assumption is here again supported by the fact that the transpiration is extremely low during the night hours, although the wind is blowing.

If transpiration and evaporation are largely determined by the same factors or, in other words, if transpiration is essentially a physical process, then a high correlation between transpiration and evaporation is to be expected. Reference to Table XXXVII will show that the correlation of evaporation with transpiration ranges from 0.84 to 0.95. The latter value is slightly higher than the maximum correlation (0.89) of radiation with transpiration and shows that 0.9 of the transpiration was in this instance determined by the same factors which determined the transpiration.

The relation of evaporation to transpiration is to be considered as associative rather than causative, both responding to the same environmental factors, but not necessarily in precisely the same way or to the same degree. The extent of this association furthermore depends upon the manner in which evaporation is measured. For example, the evaporation rate from a free-water surface in a very shallow tank conforms much more closely to the transpiration rate than when a deep tank is used, since the latter, on account of its large heat capacity, stores up a large amount of energy which is dissipated through evaporation during the night. It is evident that the evaporimeter must simulate the plant system as nearly as possible in absorption and heat capacity if a high degree of correlation between the two is to be attained.

LEAST-SQUARE RELATIONSHIPS BETWEEN TRANSPIRATION (OR EVAPORATION) AND ENVIRONMENTAL FACTORS

The method of least squares affords a means of determining the relative influence of the various environmental factors upon the transpiration. In these least-square reductions (Merriman, 1893, and Bartlett, 1915) the mean hourly values have been used, and it has been assumed that the relationship is linear in character—i. e., that the transpiration varies directly in proportion to the intensity of the environmental factors.

The results of the least-square reductions are presented graphically in figures 21 and 22. In all cases, the vertical component of the radiation has been employed rather than the radiation on a surface normal to the sun's rays. The reason for this will be apparent from an inspection of the radiation and transpiration charts, where it will be seen that during the early morning hours the slope of the radiation graph is much greater than that of the transpiration graph for rye, alfalfa, and amaranthus. In other words, the transpiration rate does not increase nearly as rapidly as the normal component of the radiation during the early daylight hours. In a field of grain or alfalfa, considered as a whole, it is evident that the vertical component of the radiation would alone be effective. In the case of an isolated pot of plants standing on the transpiration scale, the horizontal component would also be effective. The extent to which this enters can not be directly determined, however, and in the following discussion the vertical component has been used throughout.[1]

TRANSPIRATION AS DETERMINED BY RADIATION AND TEMPERATURE

The observed and computed transpiration graphs, the latter based on the assumption that the vertical component of the radiation and the air temperature are the primary controlling factors in transpiration, are given in figure 21. The computed equations are as follows:

For rye.....0.384 R_v+0.642 θ=T;
For alfalfa.....0.514 R_v+0.539 θ=T;
For amaranthus.....0.546 R_v+0.443 θ=T;

in which

R_v is the vertical component of radiation,
θ is the temperature rise, and
T is the transpiration.

In the above equations and in those which follow the hourly values for each term are expressed as a percentage of the maximum. In other words, the general dimensionless equation is of the form:

$$a\frac{R'_v}{R'_{v_{max}}}+b\frac{\theta'-\theta'_o}{\theta'_{max}-\theta'_o}=\frac{T'}{T'_{max}}$$

in which the primed quantities represent observed values.

[1] *Calculation of the vertical component of radiation.*—If R represents the normal component of the radiation of the sun, R_v the vertical component, and h the altitude—i. e., the angular distance of the sun above the horizon—then: $R_v=R \sin h$.

Expressing the altitude in terms of declination and hour angle (Smithsonian Institution, 1894, p. lxviii), we have $\sin h=\sin\phi \sin\delta+\cos\phi \cos\delta \cos t$,
in which

ϕ=the latitude of the place of observation;
δ=the declination of the sun—i. e., the angular distance above or below the Equator (from U. S. Navy Dept., 1912); and
t=the hour angle—i. e., the angle between the meridian plane through the place and the meridian plane through the sun.

Substituting, we have:
$R_v=R(\sin\phi \sin\delta+\cos\phi \cos\delta \cos t)$.

The daily observations are expressed on the basis of mean sun time, which introduces a slight error in the calculation of the vertical radiation component.

An inspection of the curves in figure 21 will show that the computed graph agrees with the observed transpiration graph much better in the morning than in the afternoon.[1] The computed graph always reaches its maximum in advance of the observed graph. The greater departures occur during the early afternoon and early evening. The agreement is by no means as good as is to be desired, and the graphs show clearly that transpiration can not be completely accounted for on the assumption

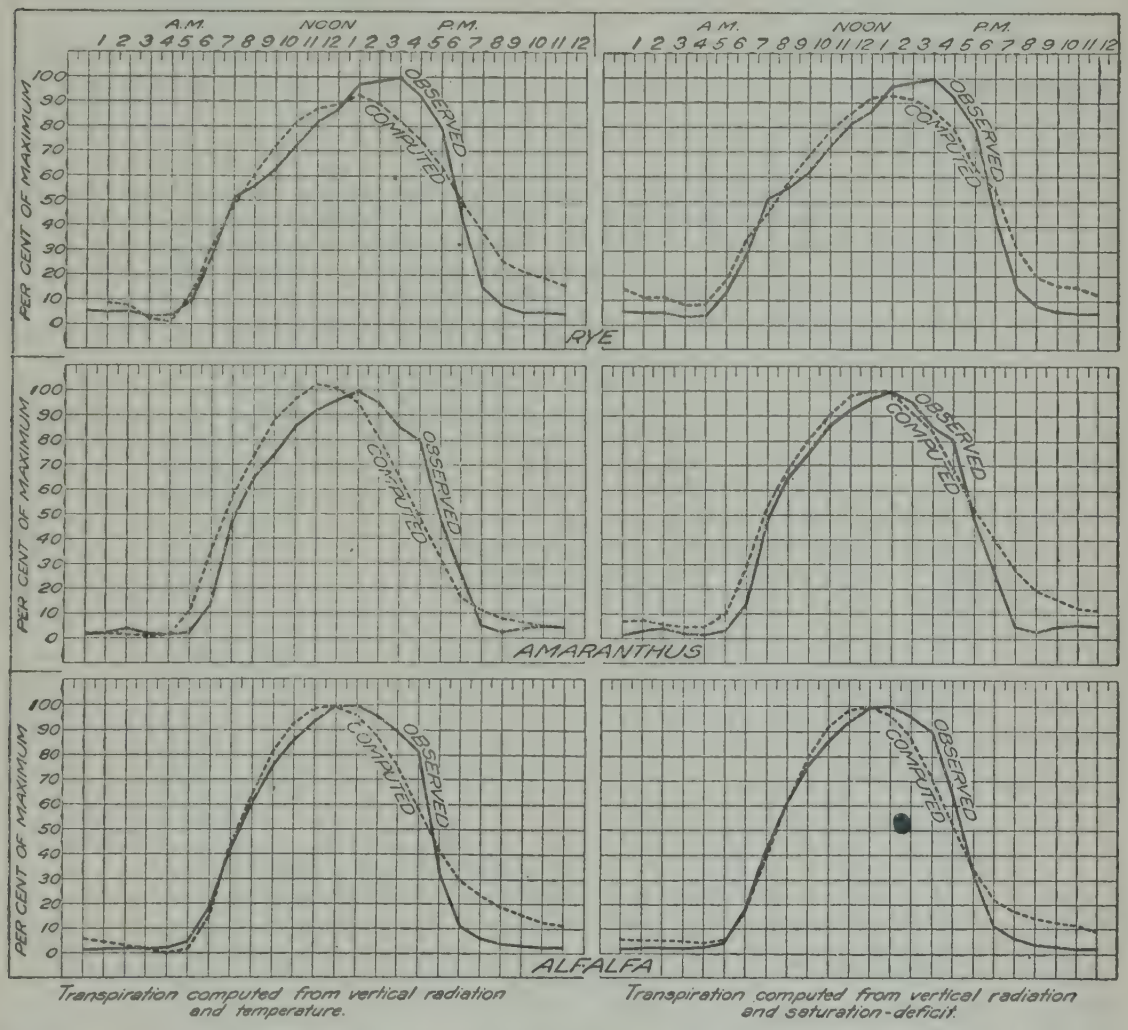

FIG. 21.—Graphs showing the observed transpiration with that computed from vertical radiation and temperature (on the left) and from vertical radiation and saturation deficit (on the right).

that the vertical component of radiation and the rise in temperature are the controlling factors.

The relative values of the computed coefficients are of interest. In the case of alfalfa, the radiation is weighted 0.97 relative to temperature; amaranthus, 1.23; and rye, 0.60. In this connection it should be recalled that rye shows a sudden change in the slope of the transpiration graph in the morning, differing markedly from alfalfa and amaranthus in this respect.

[1] Since preparing figures 21 and 22 a recalculation based on more exact determinations of the vertical component of radiation has given computed values of transpiration and evaporation which are in somewhat closer agreement with the observed values during the daylight hours than those indicated in the charts. The coefficients in the equations are based upon the revised calculation.

TRANSPIRATION AS DETERMINED BY RADIATION AND SATURATION DEFICIT

Corresponding graphs based upon vertical radiation and saturation deficit are also given in figure 21. The values for the latter term are computed from the mean hourly wet-bulb depression and the corresponding hourly air temperatures. The resulting equations follow:

For rye ..... $0.455\ R_v + 0.622\ D = T;$
For alfalfa ..... $0.538\ R_v + 0.553\ D = T;$
For amaranthus ..... $0.538\ R_v + 0.481\ D = T;$

in which D represents the saturation deficit expressed as a percentage of

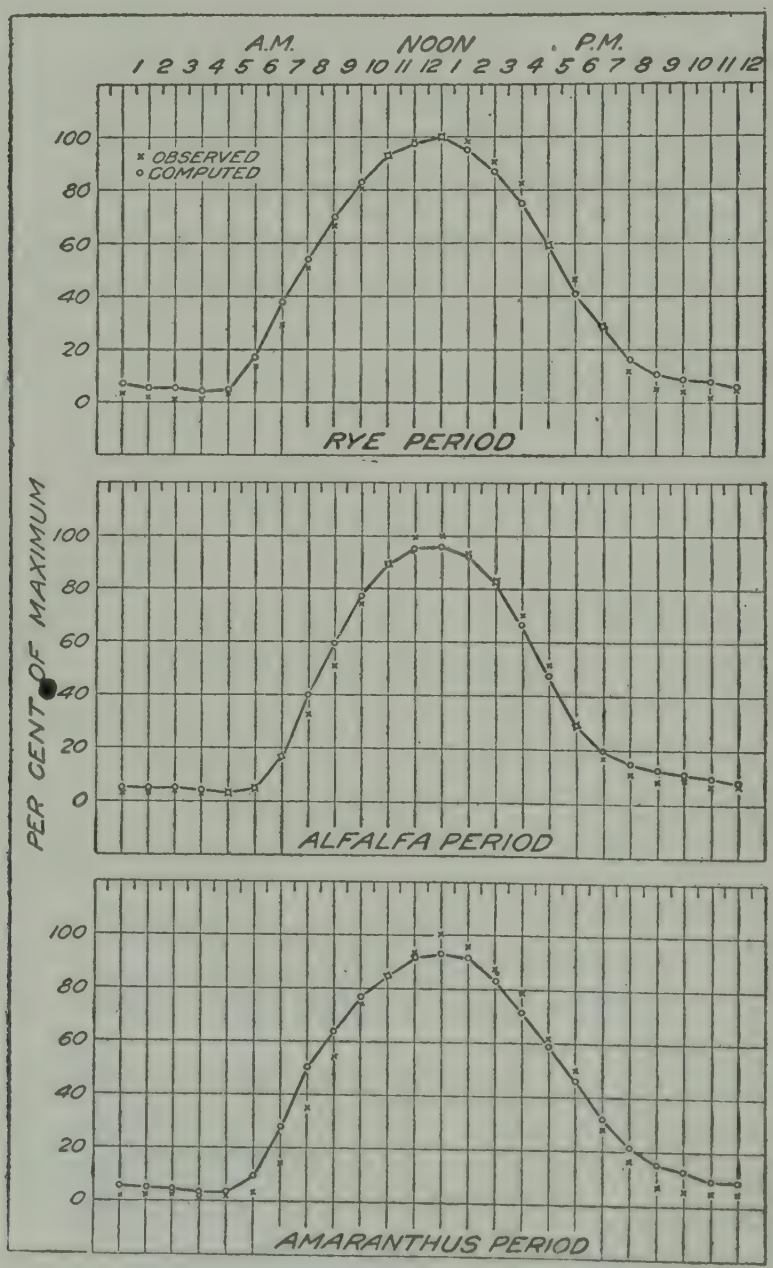

FIG. 22.—Graphs showing the observed evaporation with that computed by least-square methods from the vertical component of radiation and the saturation deficit.

the maximum, and the other symbols have the same meaning as before. An inspection of the graphs shows them to be similar in form to those computed from radiation and temperature. The coefficients are also

similar to those of the former series. The ratio of the radiation to the saturation-deficit coefficient for the different plants is as follows: Rye, 0.73; alfalfa, 0.97; amaranthus, 1.12. The equation for rye again shows the radiation to have the lesser influence of the two factors considered, while in the case of the other two plants, the radiation has an equal or greater influence. The equations for the latter plants are in fair agreement, but in all cases discrepancies occur between the observed and computed curves, particularly during the early afternoon and early evening hours.

EVAPORATION AS DETERMINED BY RADIATION AND SATURATION DEFICIT

The evaporation rate from the shallow, blackened tank for the three transpiration periods just considered has also been computed, assuming the vertical radiation and the saturation deficit to be the controlling environmental factors. The observed and computed evaporation graphs are given in figure 22. The agreement during the rye and alfalfa periods is very satisfactory, but during the amaranthus period the departures are greater. The evaporation equations for the several periods are as follows:

Rye period ..... $0.787\ R_v + 0.292\ D = E;$
Alfalfa period ..... $0.680\ R_v + 0.360\ D = E;$
Amaranthus period ..... $0.563\ R_v + 0.411\ D = E;$

in which E represents the evaporation expressed as a percentage of the maximum.

It will be observed that the radiation has a preponderating influence in each instance.

DISCUSSION OF LEAST-SQUARE REDUCTIONS

The least-square reductions again emphasize the fact that the transpiration response to changing environmental conditions is not the same for different plants. In other words, the distribution of the transpiration loss through the day varies with different plants. Furthermore, the distribution of the transpiration loss differs from the distribution of the evaporation loss from a shallow tank. As a whole, the agreement between observed and computed evaporation is much closer than between observed and computed transpiration. Either some factor operative in transpiration yet remains to be accounted for or the transpiration system changes its coefficient during the day. The latter condition may result from stomatal control or through the inability of the plant to secure sufficient water to maintain complete turgidity during the day. The fact that the evaporation on clear days can be satisfactorily accounted for from a consideration of radiation and saturation deficit indicates that the essential environmental factors have been considered and suggests that the outstanding differences between observed and computed trans-

piration are due to differences in the plants or to some change in the plant as the day progresses.

It is probable that plants differ also in their response to solar energy, the absorption coefficient of different plants not being the same, while the dissipation of the energy absorbed is quite different in different plants. In other words, the ratio of the energy dissipated through transpiration and lost by the plant through emissivity is not the same for all species. Such changes probably occur also in the same plant during the daily cycle, which would modify the transpiration coefficient irrespective of the changes in physical conditions.

SUMMARY

This paper deals with measurements of transpiration on clear days at Akron, Colo., in relation to environmental factors. The plants, which included wheat, oats, rye, sorghum, alfalfa, and amaranthus, were grown in large sealed pots of the type used in water-requirement measurements, containing sufficient soil (about 115 kgm.) to enable the plants to make a normal growth. The transpiration was determined by weighing, four automatic platform scales recording each 20-gm. loss being used for the purpose. Automatic records were simultaneously made of the radiation intensity, the air temperature, the depression of the wet-bulb thermometer, the evaporation, and the wind velocity. The radiation intensity and the wet-bulb depression were measured by differential telethermographs, and the evaporation rate from a free-water surface was determined by mounting a shallow, blackened evaporation tank 3 feet in diameter on an automatic platform scale.

Composite graphs are presented, showing the mean hourly transpiration rate for each of the plants considered, together with the mean hourly values of the radiation, air temperature, wet-bulb depression, and wind velocity for the transpiration period and also the mean hourly evaporation rate. On the basis of the form of the curves the transpiration graphs may be grouped into two classes having characteristic features. The cereals show a marked change in the slope of the transpiration graph in the forenoon unaccompanied by corresponding changes in the environmental factors. On the other hand, the forage plants and amaranthus give little or no indication of such a change. This flattening of the graphs in the case of the cereals appears to be due to some change in the plant, resulting in a reduction in the transpiration rate below what would be expected from the form of the curve during the early morning hours.

The hourly transpiration rate of the cereals on clear days increased steadily, though not uniformly, from sunrise to a maximum value, usually reached between 2 and 4 p. m., after which it fell rapidly to the night level. The transpiration graphs for sorghum, alfalfa, and amaranthus were somewhat more symmetrical with respect to midday, reaching

their maximum between noon and 2 p. m., after which they fell approximately with the radiation.

The transpiration during the night at Akron is very low, being only 3 to 5 per cent of the transpiration during the daylight hours.

The radiation graphs are practically symmetrical with respect to noon, showing that the days selected were relatively clear. When all the mean hourly values are expressed as a percentage of the maximum, the radiation intensity rises in advance of the transpiration (and in advance of all the other environmental factors as well) and falls either in advance of the transpiration or with it, depending on the plant considered. Radiation then may be looked upon as the primary causative factor in the cyclic changes.

The air temperature and wet-bulb depression graphs are very similar in form, since the latter can be determined from the former on days in which the absolute humidity of the air is not changing. The transpiration graphs usually rise and always fall in advance of air temperature.

The evaporation graph from the shallow, blackened tank (water approximately 1 cm. in depth) is similar in form to the graph representing the vertical component of radiation. This is to be expected, since only the vertical component would strike the horizontal water surface. The evaporation graph rises and falls with, or slightly later than, the vertical component of radiation.

Computation of the correlation coefficients between transpiration and the various environmental factors shows the radiation, air-temperature, and wet-bulb depression to be correlated with transpiration approximately to the same degree. The correlation coefficients of transpiration with radiation range from 0.82 to 0.89; with temperature from 0.77 to 0.86; and with wet-bulb depression, from 0.75 to 0.85. These figures show the intercorrelations existing among the environmental factors, since the sum of the squares of the coefficients of independent causative factors influencing transpiration can not exceed unity. If radiation is taken as the primary causative factor, the correlation coefficients show that 0.67 to 0.77 of the transpiration on clear days under Akron conditions is determined by the radiation intensity.

If the environmental factors are considered as independent, their relative influence on transpiration may be determined by the method of least squares. In the case of alfalfa and amaranthus, the vertical component of radiation and the temperature enter into the determination of transpiration in the ratio of 1 to 1, approximately; and the corresponding ratios for vertical radiation and saturation deficit are approximately the same. On the other hand, in the case of rye, the radiation by this method of reduction shows less influence than either temperature or saturation deficit on the transpiration rate, which from 9 a. m. to 2 p. m. shows a marked departure from the graph indicated by the transpiration during the early morning hours.

Least-square reductions of the dependence of transpiration upon radiation and air temperature or upon radiation and saturation deficit do not account entirely for the observed transpiration, although a satisfactory agreement between computed and observed evaporation is obtained by the use of these environmental factors. This indicates that the plant undergoes changes during the day which modify its transpiration coefficient. In other words, our results support the conclusion of other investigators that plants under conditions favoring high evaporation do not respond wholly as free evaporating systems, even if bountifully supplied with water and no visible wilting occurs.

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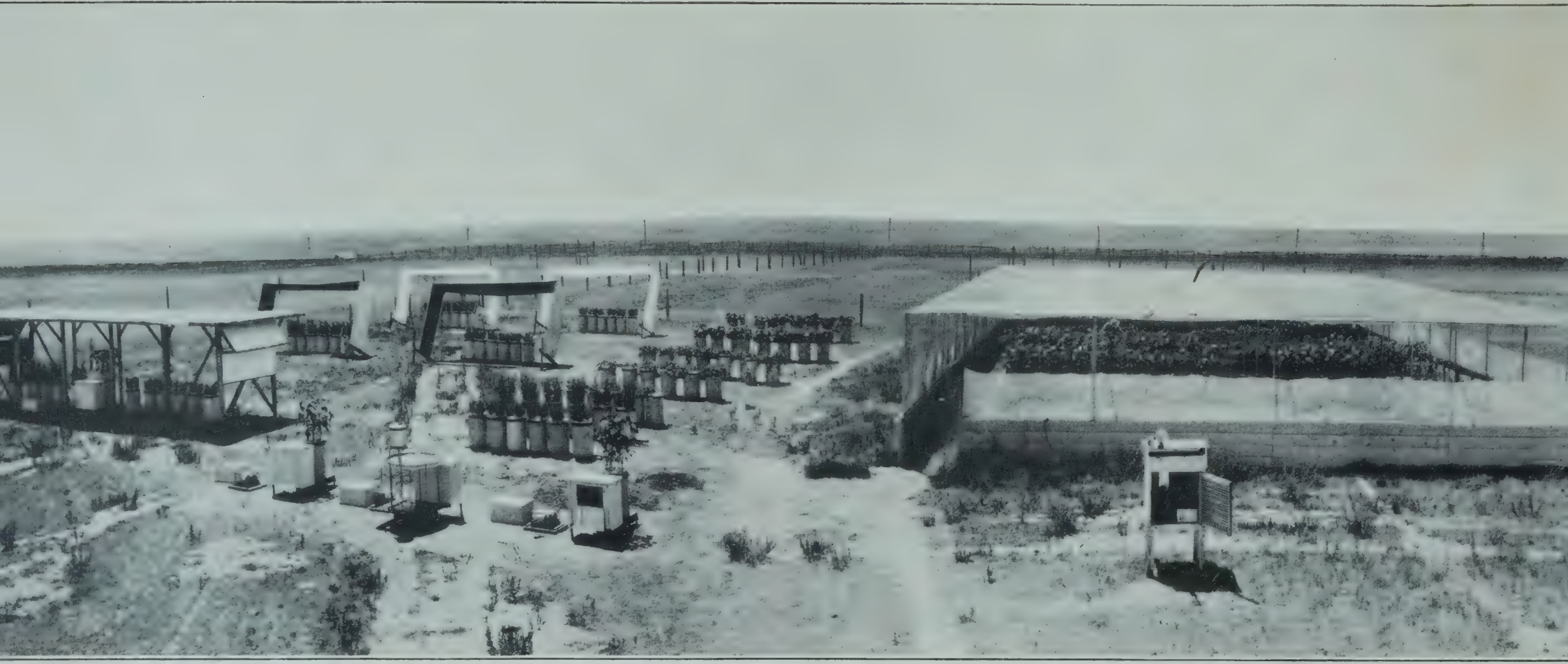
PLATE LIII

General view of the water requirement and transpiration experiments at Akron, Colo., on July 8, 1913. The large, screened inclosure in which the transpiration measurements were made in 1912 is shown at the right. The small instrument shelter in the foreground contained differential thermographs for measuring wet-bulb depression and solar radiation. The glass envelope surrounding the bulb of the radiation instrument may be seen on the top of the instrument shelter. At the left in the foreground is shown balance A, the front of the box open, and the recording device uncovered at the left. This balance is carrying a pot of sunflower. The next balance, B, carries the evaporation tank; balance C, another sunflower pot; and balance D, under the shade at the left, a third sunflower pot. The exposure of balances A and B, as used in the 1913 and 1914 determinations, is shown in this illustration.



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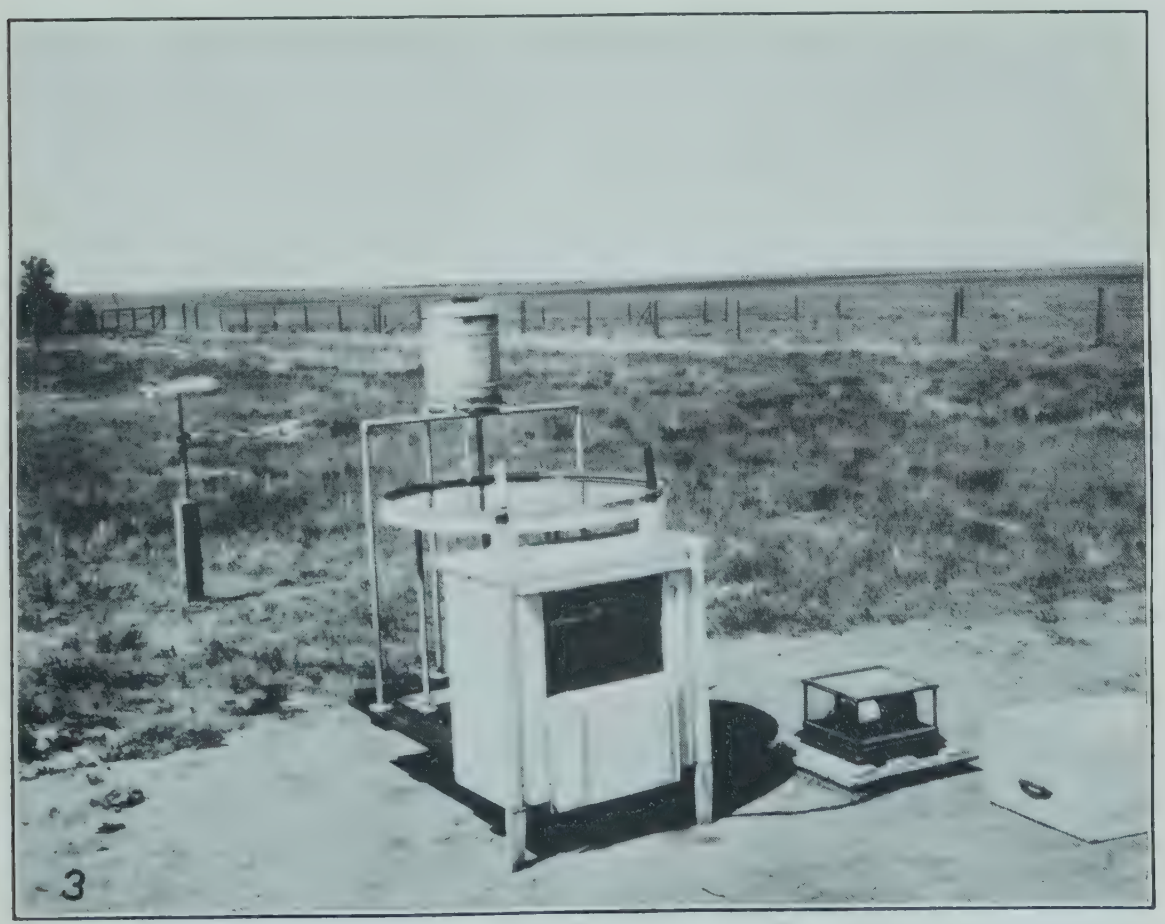
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EFFECT OF NATURAL LOW TEMPERATURE ON CERTAIN FUNGI AND BACTERIA

By H. E. Bartram,

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The effect of the very intense cold of northern winters on the life and viability of fungi and bacteria does not seem to have been tested extensively, yet its importance in checking the spread of plant infections from these sources would appear to be very great.

Wolf [1] has shown that certain parasitic and saprophytic fungi remain present and alive in Nebraska orchards during autumn, winter, and spring. The majority of the species are saprophytic, the more common ones being *Alternaria* spp., *Cladosporium* spp., and *Penicillium expansum*. Only one parasite, the cause of leafspot, was present in abundance regardless of temperature. He found more spores in the air in neglected orchards than in well-cared-for ones and also found them to be much more abundant everywhere than commonly has been supposed. All his determinations were made by exposing plates at various places in the orchard and then carefully studying and determining the colonies after they had developed.

In the present study certain known fungi and bacteria were exposed in pure cultures to the low temperature of the winter months. The organisms were started upon nutrient agar in test tubes—i. e., allowed to grow at laboratory temperature for about one week after inoculation—and then these cultures were placed in a corncrib where there was a free circulation of air, but where they were protected from the rain and snow.

The tubes were inoculated between December 10 and 16 and were exposed in the outhouse on December 21, with the exception of *Actinomyces organicus*, which was not exposed until December 31. The cultures were undisturbed throughout the winter, during which time a minimum temperature of $-24°$ C. was reached. The medium did not dry up to any extent, but was rather moist when brought into the laboratory, as the frequent freezings and thawings seemed to impair the solidifying power of the agar.

On April 14 the cultures were brought into the laboratory and tested immediately for vitality. This was done by transferring part of the exposed culture to fresh nutrient-agar slants and allowing the new inoculations to grow at room temperature. In all cases except one the response to fresh agar was soon evident, but in the case of *Actinomyces*

[1] Wolf, F. A. The prevalence of certain parasitic and saprophytic fungi in orchards, as determined by plate cultures. *In* Plant World, v. 13, no. 7, p. 164–172, fig. 1; no. 8, p. 190–202, fig. 4–5. 1910.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bm

Vol. V, No. 14
Jan. 3, 1916
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chromogenus the organism was probably killed by the low temperature. A large proportion of the conidia of both strains of *Sclerotinia cinerea* were found to be capable of germination. Table I gives the organisms and materials used and the results obtained.

TABLE I.—*Results of tests for vitality of various organisms after exposure to low temperatures (1912–13)*

Organism.	Medium.	Response of the mycelium.
Cephalothecium roseum	Synthetic agar	Excellent growth in 2 days, with production of spores.
Sclerotinia cinerea (Vermont culture).	do	Excellent growth in 36 hours; many conidia produced.
Alternaria solani	Lima-bean agar	Good growth after 2 days.
Cylindrosporium pomi	Synthetic agar	Good growth after 6 days; slow to start.
Sphaeropsis malorum	do	Slow growing at first; very good later.
Fusarium sp. of conifers	do	Excellent growth in 5 days over entire slant. Two trials needed to get results.
Glomerella rufomaculans	do	Started after 1 day and grew quickly.
Sclerotinia cinerea (culture from New Jersey Experiment Station).	do	Very good growth in 5 days.
Plowrightia morbosa	do	Excellent growth after 1 day.
Venturia inequalis	do	Good growth in 5 days with fruiting. Two trials necessary to get results.
Actinomyces organicus	Plain agar	Good growth in 2 tubes in 6 days.
Actinomyces chromogenus	do	No growth after a month. No growth on second trial.

The results secured during the winter of 1912–13 were so encouraging that further trials were made the following winter. Several organisms not tested previously were exposed with those first used, and the varieties used the first winter were tested on different media.

Since organisms in nature would be necessarily in a dry state during the winter and without much, if any, nourishment, it was the aim of the author to imitate for his pure cultures these conditions so far as possible. Accordingly, dry cultures of the various fungi chosen for this work, as well as the cultures on nutrient media, were exposed during the winter of 1913–14. These dry cultures were made by removing the growth of the fungus from the surface of the agar with a sterile needle and placing it in an empty, plugged, sterile test tube. A little of the agar was necessarily carried over with the fungus, but not enough to supply it with moisture or food for any length of time. In the case of the bacteria, some of the material from an agar slant was swabbed out with pieces of sterile cotton and placed in plugged, sterile test tubes. All of the cultures thus transferred were dried for 10 days in a warm closet in the laboratory before being exposed. It was expected that the question of food could be practically eliminated, while moisture was available only as it was carried by the air to the cultures.

The cultures were all prepared earlier the second season, and they were placed in the same corncrib on December 13, 1913. Along with the cultures was placed a Draper self-registering thermometer, in order that a comparative record might be kept of the temperatures to which the organisms would be exposed. This thermometer did not register accurately below -27° C., so that during the three periods when the tempera-

ture fell below that point the official records of the Weather Bureau were considered as applicable to this test. The temperature was recorded from the date of exposure to March 1, 1914.

Table II summarizes briefly the extremes of temperature in the corncrib and also gives the lowest official record during each week of exposure.

TABLE II.—*Temperature records at Burlington, Vt., during winter of 1913–14*

Date.	Range in corncrib.	Lowest official record.
	°C.	°C.
Dec. 12–19, 1913	7 to −13	−14
Dec. 19–26, 1913	4.5 to − 9	− 9.4
Dec. 26, 1913–Jan. 2, 1914	− 4.5 to −23	[a] −24.4
Jan. 2–9, 1914	0 to −19	−20.5
Jan. 9–16, 1914	2 to −29	[b] −32
Jan. 16–23, 1914	− 4 to −22.8	−23.3
Jan. 23–30, 1914	7 to −20.5	−22
Jan. 30–Feb. 6, 1914	4.5 to −14	.....
Feb. 6–13, 1914	2.8 to −26.6	[c] −30
Feb. 13–20, 1914	− 4 to −26	[d] −27.7
Feb. 20–28, 1914	10 to −23.3	[e] −25

[a] Jan. 1. [b] Jan. 14. [c] Feb. 12. [d] Feb. 16. [e] Feb. 25.

Tests were made of the vitality of the cultures on January 17, February 21, and March 27. These tests were made by transferring some of the growth from duplicate tubes of all the exposed cultures to fresh media of corresponding kind and holding at room temperature (19 to 22° C.) for several days. An abundance of tubes had been prepared, so that when the transfers showed no growth at the end of seven days two more exposed tubes could be brought in and tested. It will be noted that the first test for vitality was made on January 17, immediately following the extremely cold weather of January 13 and 14, when the official record was −30° and −32° C., respectively. Many of the organisms had withstood temperatures of −24° the previous winter, so it was not thought necessary to test any of them until they had experienced more severe cold. In Table III the results of these tests are summarized. Each sign used indicates the response of one culture; the plus (+) signs indicate growth, and the minus (−) signs mean that the culture was dead; "c" denotes contamination of the culture.

TABLE III.—*Results of tests for vitality of various organisms after exposure to low temperatures (1913–14)*

Organism.	Medium.	Date.	Result.	Date.	Result.	Date.	Result.	Date.	Result.
Sclerotinia cinerea	Synthetic agar	Jan. 17	+ +	Feb. 21	+ +	Mar. 27	+ +	Apr. 13	+ +
	Dry synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
Alternaria solani	Synthetic agar	17	− − − −	21	− − − −	27	− −	15	− − −
	Dry synthetic agar	17	+ +	21	+ +	27	− − + +	15	+ +
	Lima-bean agar	17	+ +	21	+ +	27	+ +	15	+ +
Cylindrosporium pomi	Synthetic agar	17	+ +	24	+ +	27	+ +	13	+ +
	Dry synthetic agar	17	− − − −	24	− − − −	27	+ − − −	13	− − − −
Sphaeropsis malorum	Synthetic agar	17	− − − −	24	− − − −	27	− − − −	13	− −
	Dry synthetic agar	17	+ +	24	− − − −	27	− − + +	13	+ +
Fusarium sp. of conifers	Synthetic agar	17	− − − −	24	− − − −	27	− − − −	13	− − − −
	Dry synthetic agar	17	+ +	24	− − − −	27	− − − −	13	− − − −
	Lima-bean agar	17	+ − − −	24	− − − −	27	− − − −	13	− − − −
Glomerella rufomaculans	Synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
	Dry synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
Plowrightia morbosa	Synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
	Dry synthetic agar	17	+ +	21	+ +	27	c c − −	13	− − + − − +
Venturia inequalis	Synthetic agar	17	+ +	24	+ +	27	+ +	13	+ +
	Dry synthetic agar	17	+ +	24	+ +	27	− − + +	13	− + +
Cephalothecium roseum	Synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
	Dry synthetic agar	17	+ +	21	+ +	27	+ +	13	+ +
Colletotrichum Lindemuthianum	Synthetic agar	17	− − − −	21	− − − −	27	− − − −	13	− −
	Dry synthetic agar	17	− − − −	21	− − − −	27	− − − −	13	− −
	Lima-bean agar	17	+ +	21	− − − −	27	− − − −	13	− −
	Dry Lima-bean agar	17	+ +	21	+ + + +	27	− − − +	13	+ +
Ascochyta colorata	Synthetic agar	17	+ +	24	− − + +	27	+ +	14	+ +
	Dry synthetic agar	17	+ +	24	− − + +	27	− − +	14	− + +
	Lima-bean agar	17	+ +	24	+ +	27	+ +	14	+ +
	Dry Lima-bean agar	17	+ +	24	+ +	27	+ +	14	+ +
Phytophthora omnivora	Lima-bean agar	17	+ +	28	+ +	30	− −	15	+ + + +
	Dry Lima-bean agar	17	− − − −	28	− −	30	− −	15	− − − −
Pseudomonas campestris	Plain-agar slants	17	− −	Mar. 2	− −	30	− −	10	− −
	Dry cotton wads	17	+ +	2	c c − −	30	c − − −	10	c − − −
Bacillus melonis	Plain-agar slants	17	+ +	2	+ +	30	+ +	10	+ +
	Dry cotton wads	17	− −	2	c c − −	30	c − − −	10	c − − −
Actinomyces chromogenus (*Oospora scabies*).	Plain-agar slants	17	c − − −	2	− −	30	c − − −	10	− − −
	Dry cotton wads	17	− −	2	− −	30	c − − −	10	− − −
Actinomyces bovis	Plain-agar slants	17	− −	2	− −	30	− −	10	− −
	Dry cotton wads	17	+ +	2	− −	30	c c − −	10	− −
Actinomyces chromogenus	Plain-agar slants	17	+ −	2	− −	30	c − − −	10	− −
	Dry cotton wads	17	+ +	2	− +	30	+ +	10	+ +
Bacillus typhosus	Plain-agar slants	17	+ + + +	2	− −	30	c c − −	10	− − − −
	Dry cotton wads	17	+ +	2	c c c −	30	c − − −	10	− − − −

If the results of the exposures of these organisms to low temperature are summarized, it will be noted that five fungi, *Sclerotinia cinerea*, *Cephalothecium roseum*, *Glomerella rufomaculans*, *Venturia inequalis*, and *Ascochyta colorata*, lived over winter under all conditions of exposure; while four others, *Alternaria solani*, *Cylindrosporium pomi*, *Plowrightia morbosa*, and *Phytophthora omnivora*, lived over on some media but not on others. One fungus, *Fusarium* sp. of conifers, succumbed to the low temperature, while two others, *Colletotrichum Lindemuthianum* and *Sphaeropsis malorum*, were so weak that only under very favorable conditions would they respond to fresh media. Only two of the six kinds of bacteria exposed can be safely said to have survived—*Bacillus melonis* and *Actinomyces chromogenus*. Transfers from exposed cultures of *B. melonis* were found to agree in all distinctive characters with those given by Giddings. It is to be noted that this organism forms no spores. The growth of transfers from exposed cultures of *Actinomyces chromogenus* was very characteristic and hardly mistakable for any other organism. *In* regard to the other bacterial cultures, it may be said that they were more or less contaminated during the exposure; and although some of the transfers from them resemble the original growth, this was not well enough marked to prevent all suspicion. On the whole, the various organisms seem to withstand exposure better in a dry condition than when food and moisture are present.

Thinking that some of the organisms might die from natural causes other than the exposure to low temperature, the author retained part of the culture made for this test indoors as a check. They were kept in a cool room (14 to 20° C.) throughout the winter and tested for vitality late in March, 1914. In practically every case these cultures were living at that time, and no organism given in Table III can be said to have died otherwise than by exposure to low temperature.

No entirely satisfactory explanation has been offered as yet of the changes which take place in fungi and bacteria during or after exposure to extreme cold. The results obtained by the author throw little or no light on the manner of the freezing nor on the subsequent death. The present work is a record of the fact that certain fungi and bacteria are able to withstand extreme cold, while others succumb to it, but does not attempt to advance any theory as to the internal changes which contribute to the weakening or death of the organisms thus tested.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., JANUARY 10, 1916 NO. 15

EFFECT OF COLD-STORAGE TEMPERATURES UPON THE MEDITERRANEAN FRUIT FLY

By E. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant, Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

INTRODUCTION

Since the introduction of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) into the Hawaiian Islands and the subsequent quarantines against Hawaiian fruits, the problem of the fruit grower in these islands has been how to use his fruit to advantage at home. Many host fruits of the fruit fly are ruined long before they are suitable for either the table or storage. There are, however, other fruits, such as the avocado (*Persea gratissima*) and certain varieties of mangos (*Mangifera indica*) and star-apples (*Chrysophyllum cainto*), which, while often becoming too badly infested to be of use if left to ripen normally upon the tree, become infested so late in their development that they may be preserved for commerce if they respond favorably to cold storage, and if such cold storage kills whatever stages of the fruit fly may be present in the fruit when picked.

The experimental work reported in this paper was undertaken primarily with the hope that it would be an aid in solving the discouraging problems of the local horticulturists. But whatever its value in this direction, it now appears that the results may be of much greater commercial importance in defining the conditions under which cold-storage temperatures will kill the fruit fly in stored fruits, thus rendering them free from danger as transporters of this pest from one country to another or even from one infested district to another in host fruits.

HISTORICAL REVIEW

Cold-storage temperatures have been used in economic entomology in the past more to suspend insect activity than to cause death, except in the case of the Mediterranean fruit-fly work in Australia and Africa. The first practical use of cold-storage temperatures known to the writers was made by the manager of a large storage-warehouse company of Washington, D. C., in an attempt to find a safe method of protecting clothing from insect ravages during the warmer period of the year. At

the suggestion and with the assistance of Dr. L. O. Howard experiments were carried on to determine the effect of cold-storage temperatures upon still other insects affecting stored goods. Dr. Howard (1),[1] in a paper read before the eighth annual meeting of the Association of Economic Entomologists in 1896, discussed for the first time in professional entomological literature the important use to which cold-storage temperatures may be put in controlling insects. In 1905 Duvel (2), while investigating the storage of cowpeas (*Vigna sinensis*), found that storage at 32° to 34° F. was entirely practicable and economical in combating the common bean weevil (*Bruchus obtectus*), the cowpea weevil (*Bruchus chinensis*), and the four-spotted bean weevil (*Bruchus quadrimaculatus*).

While the work referred to above was carried on primarily to safeguard produce and stored goods from attack during certain periods when pests are active, experiments to determine the effect of cold-storage temperatures upon the Mediterranean fruit fly have been undertaken with the object of killing the various stages within the fruit. The interest in this work in Africa and Australia has grown out of the fact that the growers have sought for their surplus fruit markets in northern Europe, England, and North America, and even in South America, China, and the Hawaiian Islands. To reach these markets their fruits must be in transit a sufficiently long time for infestations overlooked at the packing houses to cause considerable decay unless the cold-storage temperature to which the fruit is subjected en route either suspends or kills chance cases of infestation.

In 1906, Fuller (3) recorded the resistance of fruit-fly larvæ in a certain lot of peaches in Natal to 40° F. for 124 days. The writers question the accuracy of this statement, as they have been unable at this temperature to keep larvæ or eggs alive for more than 22 days, in tests covering several thousand larvæ and eggs (see Table I). Fuller believes from his observation that cold storage as a method of substitution for quarantines involves considerable risk.

Lounsbury (4) states in 1907 that experiments conducted by him in South Africa indicate that a temperature of 38° to 40°, continued for three weeks, is sufficient to insure the death of all fruit-fly larvæ in infested fruit, that two weeks at such a temperature causes considerable mortality, and that one week is thoroughly ineffective. In 1908, in a second paper (6), he records no living larvæ among 511 specimens found in peaches held for 21 and 27 days at 38° to 40°. It is his belief that the storage temperature necessary for the preservation of fruit in transit from Africa to countries of the Northern Hemisphere and to America is amply low to effect the extinction of all life in larvæ and eggs of the fruit fly contained within it.

Hooper (5) recorded in 1907 in West Australia that he had found that larvæ and eggs of the fruit fly could not resist temperatures ranging from

[1] Reference is made by number to "Literature cited, p. 665–666.

33° to 35° for more than 15 days, and advised that fruit kept within this range of temperature for three weeks would be perfectly free from living forms. His report indicates that the work was done carefully.

The work of Wilcox and Hunn (7) in 1914 has shown that such semi-tropical host fruits as the star-apple, fig (*Ficus* spp.), papaya (*Carica papaya*), mango, and avocado withstand without injury to texture or flavor a temperature slightly above 32° for from 27 days in the case of papaya to two months in the case of the avocado. Such periods at 32° are well above the margin of safety for complete mortality of the larvæ and eggs of the fruit fly.

EXPERIMENTAL WORK

In determining the effect of cold-storage temperatures upon the eggs and larvæ of the Mediterranean fruit fly, the writers have been fortunate in securing the cooperation of an ice company during 1913 and of an electric company during 1914 and 1915. At the cold-storage plants of these companies there were to be had all the facilities found in modern, well-regulated cold-storage plants. While an abundance of fruit-fly material is to be had in and about Honolulu, the writers have preferred in their work to infest in the insectary host fruits known to be previously free from attack. As no such fruits can be found in Hawaii under natural conditions, apples (*Malus* spp.) from California were used. These fruits were suspended for several hours in jars containing several hundred ovipositing fruit flies and then removed and held in the insectary for the number of days which experience had shown was necessary for the flies within to reach the stages desired for experiment. In this way larger amounts of material in definite stages could be used at one time than otherwise. While much of the data recorded in Table I was secured from fruit flies in apples, a sufficient amount, including observations on many thousands of eggs and larvæ, has been secured from fruit flies in peaches and kamani nuts (*Terminalia catappa*), as checks, to prove that there is no probability that the nature of the host fruit affects the action of temperatures.

No examination of material to determine the effect of various temperatures was made until the host fruits had been removed from storage from 24 to 48 hours. By placing the host fruits within storage the eggs and larvæ were under normal conditions. On examination the eggs were dissected out of the punctures and placed in moist chambers where all that hatched might be recorded. Larvæ found torpid though normal in color on examination within 24 to 48 hours after removal from storage invariably failed to resume activity.

THE EGG

No eggs hatch in cold storage if held at temperatures below 50° F.

A temperature of 32° proved quickly fatal to eggs. A total of 6,747 eggs were under observation. No eggs hatched upon removal from

storage after the ninth day of refrigeration. Only one egg hatched on the ninth day, and but 2 out of 2,327 removed on the seventh, eighth and ninth days. After the tenth to fifteenth days of refrigeration, 2,221 eggs were removed to warmer temperature, but none hatched. Mortality increased rapidly after the fourth day of refrigeration; thus, on the fifth day only 15 out of 735 eggs hatched. (See Table I.)

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
1	32	81	81	.....	.....	252	40	33	7
2	32	528	520	94	0	463	9	53	2
3	32	150	135	37	1	226	15	16	75
4	32	336	216	285	26	152	0	101	3
5	32	735	15	196	202	71	175	.....	.....
6	32	469	12	26	165	18	50	105	10
7	32	659	1	11	454	14	64	135	132
8	32	834	0	2	845	20	423	38	200
9	32	734	1	0	339	11	473	20	429
10	32	.....	.....	0	701	0	257	.....	.....
11	32	635	0	0	450	0	332	6	374
12	32	887	0	0	440	0	493	0	157
13	32	.....	.....	0	355	0	276	0	173
14	32	699	0	0	273	0	248	0	152
15	32	.....	.....	.....	.....	0	262	0	144
2	32–33	.....	.....	86	0	78	0	3	0
3	32–33	.....	.....	154	1	146	2	89	0
4	32–33	.....	.....	46	0	73	0	32	0
5	32–33	.....	.....	96	0	39	0	30	0
6	32–33	152	23	279	7	8	1	24	0
7	32–33	.....	.....	31	1	16	11	9	0
8	32–33	401	5	35	163	3	27	10	16
9	32–33	.....	.....	0	169	0	167	2	14
10	32–33	357	0	2	179	0	110	0	31
12	32–33	784	0	0	880	0	86	0	35
13	32–33	900	0	0	637	0	35	0	2
14	32–33	1,001	0	0	425	0	42	0	28
15	32–33	1,121	0	0	255	.....	.....	.....	.....
16	32–33	312	0	0	519	0	43	.....	.....
17	32–33	.....	.....	0	143	0	29	0	3
3	33–34	.....	.....	60	0	94	0	55	0
4	33–34	.....	.....	108	2	107	2	68	0
5	33–34	.....	.....	42	26	79	28	.....	.....
6	33–34	.....	.....	68	32	286	169	8	5
7	33–34	.....	.....	75	20	81	100	55	1
8	33–34	300	45	46	20	35	175	51	48
9	34–34	500	0	38	207	48	456	31	189
10	33–34	541	0	4	1,446	32	296	0	48
11	33–34	.....	.....	0	72	0	314	4	126
12	33–34	358	0	1	215	0	500	0	48
13	33–34	.....	.....	2	632	0	385	0	4

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
		Number under observation.	Number hatching after removal from storage.	First instar.		Second instar.		Third instar.	
				Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
14	33–34	1,035	0	0	76	0	245	0	49
15	33–34	746	0	0	710	0	301	3	154
16	33–34	1,058	0	1	763	0	65	0	53
17	33–34	513	0	0	521	0	45	0	134
18	33–34	1,000	0	♂	514	0	46	.....	.....
19	33–34	.....	.....	0	221	0	67	0	18
8	34–36	.....	.....	.....	.....	0	11	7	170
9	34–36	.....	.....	.....	.....	0	21	1	176
10	34–36	.....	.....	0	44	0	8	5	321
11	34–36	236	0	0	192	0	60	0	225
12	34–36	.....	.....	0	74	0	138	4	399
13	34–36	241	0	.....	.....	0	84	0	436
14	34–36	.....	.....	0	111	0	19	0	354
15	34–36	.....	.....	0	42	0	6	0	158
2	36	167	131	.....	.....	120	5	242	2
3	36	281	261	166	3	261	1	260	6
4	36	419	419	127	2	245	4	180	22
5	36	433	405	288	2	473	25	256	24
6	36	365	254	75	57	334	12	158	77
7	36	184	150	28	142	147	43	62	157
8	36	454	264	1	382	0	323	33	363
9	36	858	335	1	475	0	300	2	402
10	36	301	27	0	494	0	385	0	160
11	36	652	2	0	588	0	437	0	186
12	36	728	0	0	670	0	858	0	213
13	36	534	0	0	504	0	91	0	364
14	36	463	0	0	443	0	54	1	261
15	36	568	0	0	573	0	22	1	198
16	36	480	0	.....	.....	0	38	0	251
17	36	532	0	.....	.....	.....	.....	.....	.....
3	36–40	.....	.....	.....	.....	42	2	.....	.....
4	36–40	.....	.....	.....	.....	127	46	.....	.....
5	36–40	.....	.....	.....	.....	123	3	.....	.....
6	36–40	.....	.....	.....	.....	127	25	.....	.....
7	36–40	.....	.....	.....	.....	18	94	.....	.....
8	36–40	.....	.....	.....	.....	0	13	60	258
9	36–40	136	0	.....	.....	0	25	3	112
10	36–40	128	0	.....	.....	.....	.....	.....	.....
11	36–40	125	0	0	102	0	18	0	275
12	36–40	122	0	0	23	0	12	0	256
13	36–40	.....	.....	.....	.....	0	25	0	352
14	36–40	185	0	0	32	0	275	0	522
15	36–40	.....	.....	.....	.....	0	218	0	163
16	36–40	.....	.....	0	48	0	69	0	324
17	36–40	106	0	.....	.....	0	131	.....	.....
18	36–40	.....	.....	0	118	0	18	0	97
19	36–40	210	0	.....	.....	.....	.....	.....	.....
20	36–40	.....	.....	0	16	0	64	.....	.....

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	° *F.*								
10	38–40	.....	.....	38	8	19	8	10	1
12	38–40	.....	.....	4	25	26	19	36	6
13	38–40	.....	.....	3	60	15	0	.....	.....
14	38–40	.....	.....	0	36	17	40	.....	.....
15	38–40	.....	.....	15	46	5	24	.....	.....
16	38–40	.....	.....	0	99	14	148	1	25
20	38–40	.....	.....	0	42	0	39	4	3
23	38–40	.....	.....	0	43	0	84	.....	.....
25	38–40	.....	.....	0	18	0	133	0	1
28	38–40	.....	.....	0	33	0	27	0	9
30	38–40	.....	.....	0	44	.....	.....	.....	.....
2	40–45	12	12	.....	.....	.....	.....	.....	.....
3	40–45	55	19	.....	.....	.....	.....	.....	.....
4	40–45	26	0	.....	.....	.....	.....	.....	.....
5	40–45	8	3	.....	.....	.....	.....	.....	.....
6	40–45	16	12	.....	.....	.....	.....	.....	.....
8	40–45	14	7	.....	.....	.....	.....	.....	.....
9	40–45	31	17	.....	.....	.....	.....	.....	.....
11	40–45	14	1	.....	.....	.....	.....	.....	.....
14	40–45	31	1	.....	.....	.....	.....	.....	.....
15	40–45	30	0	.....	.....	.....	.....	.....	.....
17	40–45	26	6	.....	.....	.....	.....	.....	.....
19	40–45	21	0	.....	.....	.....	.....	.....	.....
20	40–45	67	2	.....	.....	37	34	80	56
21	40–45	127	0	.....	.....	79	79	138	135
22	40–45	50	0	.....	.....	107	130	187	103
23	40–45	15	0	.....	.....	.....	.....	.....	.....
24	40–45	21	0	.....	.....	92	97	160	226
25	40–45	38	0	.....	.....	68	182	125	220
26	40–45	.....	.....	.....	.....	14	281	89	88
28	40–45	.....	.....	.....	.....	30	95	106	320
29	40–45	.....	.....	.....	.....	0	9	27	208
31	40–45	.....	.....	.....	.....	1	131	57	112
32	40–45	.....	.....	.....	.....	0	161	8	201
33	40–45	.....	.....	.....	.....	0	8	4	139
36	40–45	.....	.....	.....	.....	0	290	5	318
37	40–45	.....	.....	.....	.....	0	218	7	397
38	40–45	.....	.....	.....	.....	0	345	3	393
39	40–45	.....	.....	.....	.....	0	204	7	377
40	40–45	.....	.....	.....	.....	0	42	1	385
41	40–45	.....	.....	.....	.....	0	84	0	401
42	40–45	.....	.....	.....	.....	0	112	2	330
44	40–45	.....	.....	.....	.....	0	92	0	292
45	40–45	.....	.....	.....	.....	0	39	0	200
46	40–45	.....	.....	.....	.....	0	36	1	689
						0	23	0	476

Temperatures ranging from 32° to 33° proved equally fatal, the effect on 5,055 eggs being practically identical with that recorded for an even 32° F. Thus, no eggs hatched from batches removed between the ninth

and sixteenth days of refrigeration, although 4,475 were under observation. Only 5 eggs hatched out of 401 removed on the eighth day, and 23 out of 152 removed on the sixth day.

Temperatures ranging from 33° to 34° proved fatal after the eighth day; 45 eggs out of 300 removed on the eighth day hatched. No eggs hatched out of 6,051 removed between the ninth and eighteenth days of refrigeration.

At 34° to 36° eggs were examined only on the eleventh and thirteenth days of refrigeration. No eggs hatched out of 236 and 241 removed after these periods of refrigeration.

All the eggs subjected to a temperature of 36° were not killed until after the eleventh day of refrigeration. Out of 652 eggs removed from storage on the eleventh day, 2 hatched; and out of 301 eggs removed after 10 days, 27 hatched. No eggs hatched out of 3,305 removed after from 12 to 17 days of refrigeration. No appreciable mortality occurred at this temperature until after one week.

No eggs held at 36° to 40° were examined until the ninth day of refrigeration. Out of 1,012 eggs removed in small batches daily between the ninth and nineteenth days of refrigeration, none hatched.

Only 602 eggs were used for refrigeration at 40° to 45°. No eggs hatched after a refrigeration of 21 days. Two eggs out of 67 refrigerated for 20 days hatched on removal to the laboratory. No eggs hatched of those removed after 21 to 25 days of refrigeration.

THE LARVA

Larvæ in the third instar proved more resistant to cold than larvæ in the first and second; and all instars are generally more resistant to low temperatures than are the eggs. (See Table I.)

A temperature of 32° F. was found fatal to larvæ of the first instar after the eighth day of refrigeration; 2,558 larvæ removed after refrigeration from 9 to 14 days were found to be dead. The data in Table I show that 2 out of 845 were alive on the eighth day of refrigeration and only 11 out of 454 on the seventh day. This temperature did not appear to affect the first-stage larvæ appreciably until after the fifth day of refrigeration. Larvæ of the second instar failed to live after the ninth day, and very few lived that long; but 11 out of 473 and 20 out of 423, respectively, were alive after the eighth and ninth days of refrigeration. All of 1,868 second-instar larvæ were found dead on removal from storage after the tenth to fifteenth days of refrigeration. Only 6 out of 332 larvæ of the third instar were alive on the eleventh day of refrigeration; 626 larvæ removed after 12 to 15 days of refrigeration were found dead.

A temperature of 32° to 33° had practically the same effect upon 5,352 larvæ as did 32°.

Temperatures ranging from 33° to 34° did not prove entirely fatal to the first-instar larvæ until the seventeenth day of refrigeration; one larva out of 763 was alive on the sixteenth day. This was very exceptional and demonstrates the value of using an abundance of material and of continuing examinations after all larvæ seem to have been killed. Only 4 out of 1,446 were alive after 10 days of refrigeration; 1 out of 215 after 12 days, and 2 out of 632 after the thirteenth day of refrigeration. First-instar larvæ to the number of 1,256, removed after the seventeenth, eighteenth, and nineteenth days of refrigeration, were all dead. No second-instar larvæ subjected to 33° to 34° were found alive after the tenth day of refrigeration; 1,997 removed after 11 to 19 days of refrigeration were all dead. A few third-instar larvæ subjected to 33° to 34° lived until the fifteenth day of refrigeration, but none for a longer time. After the ninth day no larvæ were found alive, except during the examinations made after the eleventh and the fifteenth days of refrigeration, when 4 out of 126 and 3 out of 154, respectively, were found alive. A study of the data in Table I shows that a temperature of 34° to 36° had practically the same effect upon 1,615 larvæ as did that of 33° to 34°.

A temperature of 36° proved fatal to first-instar larvæ after the tenth day. After the ninth day of refrigeration 1 out of 476 was found alive. No living first-instar larvæ out of 3,272 were found alive after refrigeration from 10 to 15 days. The mortality at this temperature among first-instar larvæ became very noticeable after the sixth day of refrigeration, when 57 out of 132 larvæ were found dead. No second-instar larvæ were found alive after the eighth day of refrigeration; thus, all of 2,508 removed after refrigeration from 8 to 16 days were found dead. No third-instar larva was found alive after the ninth day of refrigeration, except on the fourteenth and fifteenth days, when 1 living larva was found out of 262 and 199 larvæ examined. After the ninth day but 2 out of 404 larvæ were found alive.

Temperature, 36° to 40° F.: No examinations were made to determine the effect of this temperature on the first-instar larvæ until after the tenth day of refrigeration. Of 339 larvæ removed after refrigeration from 11 to 20 days, none was alive. No living second-instar larva was found alive after the eighth day of refrigeration; after the seventh day 18 out of 112 were found alive. All of 868 second-instar larvæ removed after refrigeration from 8 to 20 days were dead. No living third-instar larva was found after refrigeration for 10 days, 3 out of 115 being alive after refrigeration for 9 days. All of 1,989 larvæ removed after refrigeration from 11 to 18 days were dead.

Temperature, 38° to 40° F.: All of 279 first-instar larvæ removed from storage after refrigeration from 16 to 30 days were dead, 15 out of 61 being alive after refrigeration for 15 days. No living second-stage larva was found after refrigeration from 20 to 28 days. No examina-

tions were made on the seventeenth, eighteenth, and nineteenth days; on the sixteenth day of refrigeration 14 out of 162 second-instar larvæ were alive. Third-instar larvæ were found alive after refrigeration for 20 days. No examinations were made between the twenty-first and twenty-fourth days, but no living third-instar larvæ were found during examinations of larvæ after the twenty-fifth and twenty-eighth days of refrigeration.

The warmest temperatures to which fruit flies were subjected ranged from 40° to 45°. Only larvæ of the second and third instars were used. One second-instar larva was alive on the twenty-ninth day, but no living second-instar larvæ were found thereafter, although a total of 1,658 larvæ were examined after refrigeration from 31 to 46 days. One third-instar larva was alive on the forty-fifth day. All of 476 third-instar larvæ examined on the forty-sixth day of refrigeration were dead. More data at this temperature are desirable to fix the limit safely in so far as the mature larvæ are concerned. Fruit is not, however, held at such high temperature as 40° to 45° for periods sufficiently long to kill the fruit-fly larvæ; hence, the effect of these temperatures is of far less importance than that of temperatures ranging from 32° to 40°.

CONCLUSION

The data contained in this paper show that no eggs or larvæ of the Mediterranean fruit fly survived refrigeration at 40° to 45° F. for seven weeks, at 33° to 40° for three weeks, or at 32° to 33° for two weeks. They may lead to the modification of existing quarantines and encourage the refrigeration of fruit subject to fruit-fly attack. It seems reasonable to conclude that sooner or later the certification of properly refrigerated fruit will be practicable. When an association of fruit growers or a people find it financially worth while there is no reason why they can not operate a central refrigeration plant under the supervision of an official whose reputation shall be sufficient to guarantee all fruits sent out from the plant to be absolutely free from danger as carriers of the Mediterranean fruit fly.

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BIOCHEMICAL COMPARISONS BETWEEN MATURE BEEF AND IMMATURE VEAL[1]

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INTRODUCTION

Several excellent treatises on dietetics contain statements to the effect that immature veal—i. e., veal that is about 3 weeks old or less—is unfit for human food, but these statements apparently are not based upon experimental data. At least, a search of the literature showed that too few workers have studied this subject. Certain European writers say that immature veal is bad because certain American laws forbid the sale of veal less than 3 or 4 weeks of age. Conversely, the American laws were based, to some extent, at least, upon European opinion. The desirability of further experimental work was very apparent several years ago to Drs. Melvin and Mohler, of the Bureau of Animal Industry, who started the present investigation.

The following quotations are typical of the existing literature on the subject:

Thompson (1909, p. 141):[2] Veal, especially when obtained from animals killed too young, is unusually tough, pale, dry, and indigestible; but when the animals are slaughtered at the ripe age, the meat is sometimes tender, and is regarded by many as nutritious. It differs considerably from beef in flavor, and contains more gelatin and water but less fat and protein. Veal broth is nutritious, and affords a wholesome variety in the dietary for the sick. When too much is given it may excite diarrhea. Veal is much more used for invalids in Germany than elsewhere, although it figures less conspicuously in hospital dietaries there now than formerly. Bauer declares it to be more digestible than beef, but Pavy says, referring to both veal and lamb, "they are meats that it is desirable to avoid, generally speaking, in case of dyspepsia," and this opinion is prevalent in America as well as in England.

Also (p. 420): The meat of very young animals should never be eaten, and the sale of young or "bob" veal two or three weeks old is prohibited by law. It is indigestible, innutritious, and readily decomposes.

Hutchinson states (1911, p. 67–68): Veal is believed to be somewhat difficult of digestion, a belief which is confirmed by experiment, for it required two and a half hours for its digestion, as compared with two hours for beef (Jessen). The difficulty of digesting veal is somewhat surprising, for the connective tissue, though abundant, is very easily changed into gelatin. It is believed by some that the explanation is to be found in the ease with which the fibers of veal elude the teeth on mastication.

[1] The object of the present work was to ascertain whether the flesh of calves 3 weeks of age and under is or is not fit for human food. The work was begun in the spring of 1912 at the suggestion of Dr. John R. Mohler, then Chief of the Pathological Division, Bureau of Animal Industry, and continued with little interruption up to the fall of 1914. The writer is indebted to Dr. Mohler for his very effective interest in the work and for many valuable suggestions.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 708–711.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 15
Jan. 10, 1916
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No experimental data on the digestibility of veal were found in the writings of Bauer (1885) and Pavy (1881), referred to by Thompson; there was nothing more than the statement that veal was not easily digested.

Although the above-mentioned work of Jessen (1883) was apparently done as accurately as the technic of that day permitted, it was far from conclusive, partly because the experiments were not numerous enough and partly because biochemical methods for accurately measuring the speed of digestion from one stage to another had not been developed. In fact, the fundamental data regarding the chemical nature of the digestive process and of the various digestion products of proteins were just then being studied. In the same volume with Jessen's work is one of the early works of Kühne and Chittenden (1883), describing the then little-known bodies resulting from the digestion of proteins.

Undoubtedly, the alleged indigestibility of veal was a belief perpetuated by repeated quotation either of experiments too old to be conclusive or of opinions expressed elsewhere.

WORK OF PREVIOUS INVESTIGATORS

With the exception of the works of Fish (1911; 1912; 1914), very little direct experimental work was found, although a careful search of the literature was made. An excellent discussion of the subject by Fish and other workers has been published by the American Veterinary Medical Association (1912). In his earlier work Fish obtained data on the amount of moisture in immature veal and in beef, also on the freezing point of the juice from the tissues and on the specific gravity of such juice. He conducted dietetic experiments in which 7 families of 20 persons of various ages received immature veal as part of their diet. The following extracts are from his reports:

> All partook of the veal and appeared to relish it. None of the families reported any disturbance of any of the bodily functions; the health was apparently normal and each family was ready to receive a portion whenever another carcass was available. (1911, p. 139.)
>
> The claim that the flesh of very young animals has a laxative effect upon human beings (Walley) has not been verified in the present experiments. (1912, p. 148.)

In a recent work Fish found that beef and immature veal digested with equal speed in pepsin-hydrochloric acid (1913, p. 64). This last observation is in accord with that of Langworthy and Holmes (unpublished), who found that both immature veal and market veal, when fed to men as part of their diet, have practically the same coefficient of digestibility as beef—i. e., 93 per cent.

Sparapani (1914) studied the toxicity, or the alleged toxicity, of fetal flesh. From his results he concluded that bovine fetal serum was less toxic than adult serum—i. e., more fetal serum was required to kill a rabbit than adult serum when injected intravenously.

EXPERIMENTAL WORK

MATERIALS

At convenient intervals a live calf, 7 days old or less, was obtained from a veterinarian in Washington, D. C., who procured the supply from farms near by. Forty-one calves were procured in this way. On 12 of these animals quantitative data were obtained; the rest of the material was used in the feeding experiments with cats. Each calf was inspected by a member of the staff of the Pathological Division. In every case, except veal sample 7, the calf purchased was found to be in good condition.

Immediately after the calf was killed, dressed, and quartered, the meat was trimmed from the bones. When the calf was intended for quantitative analytic work and for digestion experiments, care was taken to remove the muscles entire or nearly entire, so as to exclude bits of bone, tendon, etc. The whole muscles, free from adherent fat and the tough, tendinous ends, were placed in a wide-mouth 8-liter glass-stoppered bottle and kept in cold storage at or very near 1° C. (34° F.) until used.

When the calf was intended for feeding to the experimental cats, the meat was trimmed less carefully, so that adherent fat, small pieces of soft bone, etc., were included in the material stored. To this were added the liver, kidneys, spleen, lungs, and heart, all of which the cats received in their food (see p. 705). About 10 kgm. of muscle were obtained from each calf. A detailed record was made of the dates on which the calves were killed, etc., so that the age of the meat when used for the various purposes was always known.

Along with the analyses and digestions made on the veal, control determinations were made on beef. The greatest care was taken throughout the entire work to be certain that the data on beef and veal were obtained under identical conditions. Whenever a calf was killed and the veal was intended for comparative work with beef, 10 pounds of ordinary lean beef round steak were purchased in a market near by. No inquiries were made regarding the beef; it represented so much lean beef purchased at random. Soon after being brought to the laboratory the beef was carefully trimmed—i. e., fat and connective tissue were removed, leaving only the lean muscle tissue, with a few small specks of fat here and there. This was transferred to an 8-liter glass-stoppered wide-mouth bottle and kept until used in cold storage alongside the bottle containing the veal. The beef was numbered to correspond with the veal—i. e., beef sample 8 was the beef used for control work on veal sample 8.

Sometimes the comparative analyses and digestions were begun on veal and beef 1 day old—i. e., 1 day in storage—although the beef was really mature beef of unknown age. In some experiments the meats were

a month old, but in every case the age is given. Naturally, after the veal and beef had been stored for several weeks, they acquired "off odors." This was always recorded, but the meats were always used as if perfectly odorless. Veal intended for feeding to the cats was always boiled. None was rejected, no matter how unappetizing it might have been to human beings.

STANDARD SOLUTIONS AND APPARATUS

In the chemical work on the veal and beef the nitrogenous substances and the moisture content were studied. Together these constitute about 95 to 97 per cent of the weight of the meat, so that the chemical work, while not too detailed, gave information on practically all constituents except the lipins. For the large number of nitrogen determinations standard $N/5$ sulphuric acid and sodium hydroxid were used. Although all the nitrogen determinations were comparative—i. e., on veal and beef at the same time and under the same conditions—the absolute value of the standard acid was determined with the greatest care. This was done by precipitating and weighing the barium sulphate obtained from a known volume of the acid, and as an independent check on these results the acid was also standardized against pure ammonium sulphate and against pure sodium carbonate. It is perhaps true that with biological material such as meat the limit of accuracy is soon reached if ordinary care is used, and nothing is gained by taking unnecessary precautions. But because the wholesomeness of immature veal is a subject of controversy it was thought especially advisable to take too many precautions throughout the work rather than too few.

The volumetric apparatus used was standardized either by the United States Bureau of Standards or in the laboratory. A set of standardized analytic weights, a carefully calibrated Greene barometer, and a standardized thermometer from the Physikalisch-Technische Reichsanstalt (Charlottenburg, Germany), were used.

ANALYTIC DATA ON IMMATURE VEAL AND MATURE BEEF

TOTAL NITROGEN

The total nitrogen was determined on seven portions of each sample of beef and veal, of which three were made on the fresh meat, two on meat dried over sulphuric acid in vacuo at room temperature for two weeks, and two on portions dried for 12 hours at 95° C. in the hot-water oven.

No nitrogen determinations were made on veal samples 1 and 2—i. e., the first two calves—and the corresponding mature-beef samples. On veal and beef samples 3, 4, 5, 6, and 7 nitrogen determinations were made as just described. On veal and beef samples 8, 9, 10, 11, and 12

determinations were made as before, except that no portions of fresh meat were weighed for the direct determination of total nitrogen. Portions of 25 gm. each were weighed into suitable flasks and hydrolyzed by boiling with hydrochloric acid. After diluting to 250 c. c., two portions of 25 c. c. each, corresponding to 2.5 gm. of fresh meat, were pipetted into Kjeldahl flasks and the determination carried out as usual (see p. 678). In this way duplicate determinations were made on veal samples 8, 9, 10, and 11 and a single determination on sample 12. Duplicates were obtained on beef samples 8 and 11; on beef sample 10 four determinations were made and averaged, as the first two were not close enough; on beef sample 12 one determination was made. There was no beef sample 9. Veal sample 9 was compared with skim milk (skim-milk sample 2) which contained 5.29 mgm. of total nitrogen per gram of skim milk, or 0.529 per cent. Veal sample 5 was compared with beef sample 5 in some experiments and with skim-milk sample 1 in others—this contained 5.74 mgm. of total nitrogen per gram of skim milk.

All determinations of nitrogen were made by the usual Kjeldahl method, using metallic mercury, potassium sulphid, etc. Shortly after the appearance of the results of Trescot (1913), potassium sulphate was used in addition to the mercury, to assist in the oxidation. At first Congo red was used as indicator; later this was replaced by alizarin sulphonate.

The results for total nitrogen are summarized in Table I. It is apparent that the differences in nitrogen content between immature veal and mature beef are slight. The higher moisture content of the veal probably accounts for the slightly lower average figure, 3.14 per cent, as compared with 3.48 per cent for beef. The averages for the meats dried in vacuo are practically identical. For the meats dried in the hot-water oven, the average value for the veal, 14.08 per cent, is higher than that for the beef, probably because the veal dried more thoroughly—i. e., the average moisture in veal dried in vacuo was 77.08 per cent; in the hot-water oven, 77.54 per cent (see p. 683, moisture figures). The difference between the two figures for beef was not so great, the average for beef dried in vacuo being 74.18 per cent and in the hot-water oven 74.10 per cent.

TABLE I.—*Percentage of total nitrogen in meat*

Calf No.	Age of calf when killed.	Fresh.		Dried in vacuum desiccator.		Dried in hot-water oven.	
		Beef.	Veal.	Beef.	Veal.	Beef.	Veal.
	Days.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3	7	3.45	3.33	12.67	14.03	11.78	14.65
4	5	3.49	3.18	13.56	13.86	13.65	14.03
5	6	3.51	3.24	14.62	13.04	14.26	13.37
6	5	3.60	3.00	13.23	13.26	13.42	13.60
7	5	3.59	3.40	14.40	14.41	14.47	15.12
8	3	3.53	2.95	12.60	13.55	13.52	13.74
9	7	(a)	3.12	.....	.....	.....	.....
10	4	3.34	2.97	13.13	13.50	13.78	14.16
11	4	3.43	3.17	13.82	13.58	13.92	13.76
12	4	3.38	3.07	13.49	13.61	13.67	14.25
Average	.....	3.48	3.14	13.50	13.65	13.61	14.08
Number of determinations averaged	.....	24	24	18	18	18	18

[a] Skim-milk sample 2 was used instead of beef (see p. 695).

The figures for total nitrogen in dried meats (last four columns of Table I) were calculated back to the fresh basis for comparison with the figures obtained directly on the same samples of fresh meat, with the average results given in Table II.

TABLE II.—*Average percentage of total nitrogen in meat (dried meat calculated to fresh basis)*

Meat.	Fresh.	Dried in vacuum desiccator.	Dried in hot-water oven.
	Per cent.	*Per cent.*	*Per cent.*
Beef	3.48	3.46	3.46
Veal	3.14	3.15	3.19

It is apparent from Table II that the meats lost no nitrogen during the drying. (For the method of drying, see p. 683.) Benedict and Manning (1905, p. 312) found that "these meats [beef, chicken], therefore, after heating at 100° in a water oven lost from 4 to 7 per cent of the total nitrogen present." They quote similar observations by other investigators. What is important in this connection is not the mere loss of a small amount of nitrogen, which could be easily replaced in a diet, but the possibility that the lost nitrogen was present in the form of volatile amins, as suggested by Atwater (1895, p. 43). Some amins are very poisonous, and the presence of even small amounts of such bodies in immature veal would constitute a valid objection to its use. Although looked for, losses of nitrogen in the dried-meat samples were not observed. There may be two reasons for this: (1) The meats used were not decom-

posed, and, therefore, amins resulting from decomposition were absent; (2) the temperature inside the hot-water oven varied from 93° to 95° in winter to 95° to 97° in summer, and meat dried for 12 hours in this manner was not decomposed.

Another method of looking for toxic bodies was used, the veal being fed to cats (see p. 703).

The results for beef, summarized in Tables I and II, are practically identical with those generally obtained by other investigators. Thus, Davis and Emmett (1914, p. 449) found 3.624 per cent of nitrogen in beef dried at 100° to 105° C. for 20 hours, the result being calculated to the fresh basis. Their values for total nitrogen in beef are practically the same as those for either beef or veal in Table I. They found that there was but very slight loss, if any, on drying the meats at 100° to 105° as compared with the value found by the vacuum method. Richardson and Scherubel (1908, p. 1552) obtained the following results for total nitrogen in 13 samples of fresh lean beef: Maximum, 3.65 per cent; minimum, 3.34 per cent; average, 3.49 per cent. It is to be noticed that all the figures for fresh beef in Table I lie between this maximum and minimum, and the averages in both are practically identical. These investigators state (p. 1551) that—

> In nearly all the work on beef the muscular portion known as the "knuckle" to butchers was made use of on account of its size, uniformity in structure, and its freedom from fatty tissue. The knuckle is the group of muscles known as the Crural Triceps to anatomists and consists of the Rectus Femoris, Vastus Externus, Vastus Internus, and Anterior Gracilis. It was desired to experiment primarily upon the lean portion of beef, and fatty matter and gristle was trimmed away as far as possible in the preparation of the samples for analysis.

EXTRACTIVE NITROGEN

Portions of freshly hashed beef and veal, each weighing 100 gm. were extracted by heating in flasks with 800 c. c. of distilled water. The heating lasted one hour in a boiling water bath. After cooling and weighing the flasks, sufficient water was added to bring the final volume up to 1,000 c. c. of water plus 100 gm. of fresh meat. The total nitrogen was determined in duplicate 100 c. c. portions of the filtrates. Beginning with beef and veal samples 7, whenever meat was boiled for digestion experiments, control portions were boiled for extractive nitrogen. It is obvious that in measuring the amount of nitrogen going into solution by the digestion of meat, it was desirable to know the quantity of soluble nitrogen originally present.

In 100 c. c. of filtrate corresponding approximately to 10 gm. of meat, the extractive nitrogen actually titrated was equivalent to about 15 c. c. *N*/5 acid. In calculating the amount of nitrogen corresponding to 100 c. c. of filtrate, allowance was made for the moisture present in the meat—i. e., if the meat contained 75 per cent of water, the 100 c. c. of filtrate treated corresponded to 100/1,075 of the total extractive nitrogen

present in 100 gm. of meat. In 15 duplicate determinations on two portions of the same filtrate obtained from beef and veal samples 3 to 8, the average difference between duplicates was 0.26 c. c. *N*/5 acid; one set of duplicates on beef sample 4, in which the difference was 1.53 c. c. *N*/5 acid, was not included in this average; but the average of these two was included in the results in Table III. The data on skim milk were obtained by using 600 gm. of skim milk instead of 100 gm. of meat, making the proper calculated allowances for the water in the milk. The details of the precipitation of the casein, etc., are given on p. 692.

The results for extractive nitrogen are summarized in Table III. The last column gives the number of days that elapsed between the killing of the calf and the boiling of the meat. During this time the veal was in cold storage. This, of course, is not true of the beef. The beef when purchased was in all probability obtained from an animal killed from 8 to 18 days before. After being brought from the market, the beef was stored with the veal. While sample 3 of veal used in experiment 14 was 8 days old when boiled, the corresponding sample 3 of beef can be said to have been stored for 8 days, but its age is not known. For this reason the comparison between the two is not exact. For some purposes it might have been desirable to kill a mature animal on the premises and store the beef immediately, as was done with the veal. But the principal object was a comparison of the veal with meat as purchased in the market.

TABLE III.—*Percentage of extractive nitrogen in meat*

Sample No.	Beef.	Veal.	Experiment No.	Age of meat when boiled.
	Per cent.	*Per cent.*		*Days.*
3	[a] 0.456	[a] 0.534	14	8
4	.433	.508	15, 16	1, 9
5	.437	472	17, 18	7
5	[b] .0364	.472	19	[c] 18
6	.473	.448	20, 21, 22	2, [d] 13, [e] 21
7	.433	.646	23	3
7	.693	[f] 1.526	24	[e] 33
8	.505	.520	26	8
8	.610	.520	25	[g] 31
9	[h] .0615	.490	27	6
9	[h] .0754	.539	28	21
10	.466	.553	30	19
10	.495	.645	31	[d] 28
11	.455	.519	32	19
12	.437	.496	34	8
Average	.491	.530	.....	.....
Determinations averaged	12	13	.....	.....

[a] Meat hashed, kept in cold storage till next day, then boiled. All other samples hashed and boiled same day. Veal sample 5, experiment 17, was hashed and boiled the same day calf 5 was killed.
[b] Figure for extractive nitrogen in skim-milk sample 1 omitted from average.
[c] Veal had an "off odor."
[d] Beef and veal had an "off odor."
[e] Beef and veal very poor, not fit to eat.
[f] Veal sample 7, calf had white scours, figure omitted from average.
[g] Veal had no odor. Beef had slight odor of hydrogen sulphid.
[h] Figures for skim-milk sample 2 omitted from average.

With the exception of veal sample 7, all of the calves purchased were in good condition. Calf sample 7 was known to have "white scours," or diarrhea. It was plainly a sick animal and was purposely obtained. A very young kitten gained considerable weight while utilizing veal sample 7, boiled, as its sole source of nitrogen (see p. 707). The high content of extractive nitrogen in veal sample 7, experiment 23, while comparatively fresh, and its very rapid autolysis, as indicated by its appearance and still higher extractive nitrogen content in experiment 24 a month later, are very striking. The four duplicates on veal and beef samples 7 were excellent.

Hansoulle (1910, p. 122), in his report on very young veal as food, quotes Fonsny to the effect that about 60 per cent of the dry matter in meat from very young calves consists of extractives and gelatin, materials which, while digestible, are not assimilable. Hansoulle also quotes the opinions of several directors of abattoirs in Belgium and France who regard very young veal as unfit for human food, but references to experimental work are not given. After veal sample 7 had been stored for over a month, the extractive nitrogen—i. e., nitrogen soluble in water near the boiling point—amounted to 44.9 per cent of the total nitrogen in the meat. But, obviously, this was exceptional, at least for the calves used in this work. It is possible that under the conditions observed by Hansoulle the veal deteriorated rapidly and justified his strong pronouncements on the unfitness of very young veal. The data in Table III have been obtained on calves 7 days old or less when killed, the meat of which had been stored at about 34° F. (1° C.) for varying lengths of time. The differences between the figures for beef and veal are much smaller than would be expected from the statements of various writers that the elimination of excretory nitrogen in very young calves is slow. Excluding the figure for veal sample 7 in experiment 24, the average extractive-nitrogen content in fresh beef is 0.491 per cent, and for fresh veal, 0.530 per cent, with no great variations from the average. If the figure for veal sample 7 be included, the averages are 0.491 per cent for beef and 0.601 per cent for veal. The figures for beef are essentially the same as those obtained by other workers.

Richardson and Scherubel (1908, p. 1527), in their studies on cold-storage beef, extracted 100-gm. portions of fresh beef with water until 1 liter of extract was obtained from each. Determinations of nitrogen in the various forms were made on 50 c. c. portions of the extract. By adding together their figures for the amount of nitrogen present as ammonia (method 2), albumoses, and meat bases in their cold-water extract, a figure is obtained which corresponds to the figures for extractive nitrogen in Table III. The term "extractive nitrogen" is used rather loosely here, as it includes all nitrogenous substances in meat which are soluble in water near the boiling point—i. e., proteoses, peptones, amino acids, ammonia, purin bases, etc. The slight loss of ammonia due to the

coagulation of the meat and the heating in water was not determined or allowed for in the calculations; it is too small. Richardson and Scherubel (p. 1552) obtained the following averages on cold-water extracts from 13 samples of fresh beef (see p. 674): Nitrogen present as ammonia 0.010 per cent; albumoses, 0.024 per cent; meat bases, 0.071 per cent. The total, 0.405 per cent, corresponds closely to the average of 0.491 per cent for beef in Table III. It is natural that the figure in Table III should be a trifle higher, as it includes data on both fresh and cold-storage beef. The storage temperature was practically the same as that used by Richardson and Scherubel—i. e., 2° to 4° C. (36° to 39° F.). It will be noticed in Table III that while the meats were in cold storage for the periods there indicated the extractive nitrogen increased very appreciably in beef samples 7, 8, and 10 and in veal samples 9 and 10. The same probably happened in beef and veal samples 3 to 6, but data were obtained on these only when fresh.

Similar increases in extractive nitrogen were noticed by Richardson and Scherubel (1909, p. 99) in their study of the changes taking place in beef stored at 2° to 4° C. In their samples proteolysis took place more slowly than in those of Table III, probably because, as they state (p. 101), "the knuckles (weight 7 to 8 pounds) were hung in a temperature of 2° to 4° C. immediately after slaughter and were allowed to remain there during the period when analyses were made, that is for 121 days."

The meat stored for use in the present work was cut into pieces not much larger than a hen's egg of good size. Undoubtedly this treatment permitted more active autolysis and bacterial decomposition than would have taken place had the veal and beef been stored in larger masses. As previously indicated, entire muscles were dissected from the veal quarters for the sake of uniformity of composition of the muscle tissue used for analysis, etc., necessitating the storage of comparatively small pieces of meat (see p. 669).

Emmett and Grindley (1909) found that in beef stored for 22 days at 33° to 35° F. (0.5 to 2° C.) the extractive nitrogen, contrary to expectations, did not increase, but a slight increase was noticed in beef stored under the same conditions for 43 days (p. 425). It is probable that one reason for this observation is to be found in their method of preparing cold-water extracts of beef for analysis. Portions of the experimental beef weighing 30 to 35 gm. were repeatedly extracted with cold water, and the extracts after filtration were diluted to 5 liters (Grindley and Emmett, 1905, p. 663). After removing coagulable nitrogen in a 200 c. c. portion of such a filtrate, corresponding to 1.2 gm. of meat, a further partition of nitrogen was made on the very small amounts of nitrogen remaining. The unavoidable errors in analytic work become proportionately large under such conditions, and the detection of slight changes in meat stored under good conditions for short periods of time becomes difficult.

Many investigations have been made on the behavior of beef when frozen, but such results are not exactly comparable with those obtained by the foregoing investigators nor by the writer on beef stored at or near 2° C.

It is obvious that the beef and veal used in this work underwent proteolysis during the storage periods to practically the same extent. The changes that took place in the beef are entirely comparable with those observed by others in beef stored under similar conditions. The slightly higher average content of extractive nitrogen in the veal (Table III) is not regarded as physiologically significant in the present consideration of the fitness of 1-week-old veal as food. The extractives of immature veal are the same as those of mature beef (Lindsay, 1911), and the slight quantitative difference found between the 10 "bob-veal" calves and their corresponding 10 samples of lean beef (summarized in Table III) do not warrant the inference that the tissues of the very young calf are loaded with unexcreted nitrogenous waste products.

AMINO NITROGEN IN MEAT EXTRACTIVES

The hot-water extracts of beef and veal used for the determination of extractive nitrogen were also used for the determination of amino nitrogen in the nitrogenous extractives present. The figures obtained were used as blanks in those digestion experiments in which the rate of digestion was measured by the rate of formation of amino nitrogen (see p. 696). Any marked differences between the figures for beef and those for veal might have led to the detection of significant differences in their composition.

Ten c. c. of filtrate, containing the extractives from not quite 0.5 gm. of beef or veal, were introduced into the Van Slyke amino-nitrogen apparatus and the amino nitrogen determined exactly as it was determined in the digestion experiments (see p. 680). The volume of gas measured was small, ranging from 1.9 to 5 c. c. The weight of nitrogen so obtained was calculated to 1 gm. of fresh meat, and this figure was divided by the weight of extractive nitrogen in 1 gm. of meat. The results are summarized in Table IV. In experiment 27 the digestibility of veal sample 9 was compared with that of skim milk instead of beef. The amino-nitrogen determination on skim-milk sample 2 was made on 10 c. c. of diluted skim milk containing 600 gm. of skim milk diluted to 1,000 c. c. which was used for other determinations (see p. 695). In this case the amino nitrogen was derived not only from the nonprotein extractives but from the proteins as well. The amino nitrogen obtained was calculated to 1 gm. of skim milk, and this figure was divided by the weight of extractive nitrogen found in 1 gm. of skim milk by the method described on p. 695.

TABLE IV.—*Percentage of amino nitrogen in extractive nitrogen in beef and veal*

Sample No.	Experiment No.	Beef.	Veal.	Sample No.	Experiment No.	Beef.	Veal.
		Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
8.....	26	27	18	11.....	32	19	16
8.....	25	27	18	12.....	34	23	19
9.....	27	[a] 60	11				
10.....	30	22	19	Average.....		24	18
10.....	31	24	24				

[a] Figure for skim-milk sample 2; not included in the average (see p. 695).

The figures for the percentage of amino nitrogen in Table IV were obtained by single determinations on each filtrate. Duplicates on veal sample 10 and skim-milk sample 2 agreed almost exactly, which is to be expected when small volumes of nitrogen gas are obtained in this determination. This, together with the comparatively large blank on the reagents, makes the experimental error in these determinations higher than in others. Nevertheless the data have been obtained on five different calves and their control samples of beef, and the uniformly higher amino-nitrogen content in the beef extractives is probably a correct indication of a slight difference between the beef and veal. The significance of this difference, if any, requires further work for its elucidation.

DISTRIBUTION OF NITROGEN IN BEEF AND VEAL HYDROLYZED BY HYDROCHLORIC ACID

HYDROLYSIS.—The beef and veal were hydrolyzed by boiling with hydrochloric acid in 300 c. c. Jena glass Erlenmeyer flasks provided with ground-in condenser tubes 100 cm. in length. Into a weighed flask 25 gm. of meat were weighed quickly to the nearest 0.1 gm. and the exact weight noted. Two such portions of beef and two of veal were weighed from large samples of the meats freshly hashed for several determinations. To each flask 175 c. c. of hydrochloric acid (1 : 1) were added. The ratio is 35 parts of 20 per cent hydrochloric acid to 1 of protein, found by Van Slyke (1912, p. 296) to hydrolyze proteins completely after boiling for 24 hours. In all the experiments except the first with beef and veal sample 8 the hydrolytic mixture was boiled for 24 hours. Beef and veal samples 8 were boiled for 24 and 48 hours, but no differences in the results were found. A small piece of broken glass added to the material in the flask prevented bumping. After boiling the required length of time the mixtures were cooled, transferred to 250 c. c. volumetric flasks, and diluted to the mark. Portions of these mixtures were used in the following determinations.

TOTAL NITROGEN.—From each of the four flasks 25 c. c. portions of the mixture, corresponding very nearly to 2.5 gm. of meat, were pipetted into Kjeldahl flasks and the total nitrogen determined. The results obtained in this way on beef and veal samples 8 to 12 have been given in Table I.

AMMONIA.—The Boussingault-Shaffer method, as described by Berg and Sherman (1905), was used for the determination of ammonia[1] in the hydrolytic mixture. The apparatus used was, in general, similar to that used by Van Slyke (1911, p. 21).

Fifty c. c. of the hydrolytic mixture, corresponding to 5 gm. of meat, were used. It was desired to know whether the general assumption that no nitrogen is carried over by the hydrochloric-acid distillate was correct or not. For this purpose the distillates from beef, whenever obtained, were transferred to the same Kjeldahl flask, while the distillates from veal were transferred to another. The total nitrogen was then estimated in the usual manner. Distillates corresponding to 25 gm. of beef and 35 gm. of immature veal yielded in both cases less than 0.2 c. c. of $N/5$ nitrogen, indicating that none was lost during the distillations.

The distillation of the ammonia was carried out as usual for one hour in every case, during which time there appeared to be no splitting off of "cleavage ammonia," as numerous tests indicated.

In the hydrolytic mixtures obtained from beef and veal the ammonia nitrogen was about 7 per cent of the total nitrogen. Because of the small amount of ammonia actually distilled, corresponding to 5 gm. of fresh meat, or about 1 gm. of protein, the unavoidable errors in the analyses are proportionately large. The differences between six duplicates on beef and veal samples 8, 10, and 11 (Table V) varied from 0.04 to 1.33 per cent of the total nitrogen; average, 0.5 per cent. An idea of the limits of accuracy of this determination may be obtained by comparing the figures for ammonia nitrogen in casein by Van Slyke (1912, p. 297), who found 10.1 and 10.27 per cent, with those by Sherman and Gettler (1913), who found 10.0 per cent.

In order to be better able to compare the results for ammonia nitrogen, etc., in beef and veal with similar results by other workers, a sample of pure casein was hydrolyzed, using 5 gm. of casein instead of 25 gm. of fresh meat. The results obtained were: On casein hydrolyzed for 24 hours, 10.04 and 10.38 per cent, and for 48 hours, 10.55 and 10.81 per cent, of the total nitrogen present as ammonia, indicating that the technic used was essentially similar to that used by the above investigators (see p. 682).

MELANIN NITROGEN.—To the mixture remaining in the distillation flask after the removal of ammonia 3 c. c. of concentrated hydrochloric acid were added, the material transferred to a 100 c. c. volumetric flask, and diluted to the mark. This was then filtered into a second clean, dry 100 c. c. flask, and the nitrogen was determined in the melanin on the filter paper, corresponding to 5 gm. of meat, by the Kjeldahl method in the usual manner. To the figure so obtained there was added the amount of melanin nitrogen occasionally obtained by filtering the hydrolytic

[1] For excellent discussions of the various methods for determining ammonia, see Smith (1913); also Shŭlansky and Gies (1913).

mixture before any determinations were made. The filtrate was used in the determinations of amino nitrogen.

AMINO NITROGEN.—Van Slyke's (1912) apparatus and method were used in this determination in exactly the same manner for the hydrolytic mixtures, digestion mixtures, and control determinations on the reagents, leucin and pure casein. In every case the reaction between the reagents and the solution introduced into the apparatus was allowed to go on for exactly 20 minutes. Two, and sometimes three or four, determinations of amino nitrogen were made on every sample mentioned in Table V. The distribution of nitrogen was not studied in beef and veal samples 1 to 7. Two determinations on the same solution of hydrolyzed beef or veal generally differed by 2 per cent; thus, the figures obtained for veal sample 8 were 70.2 and 72.3 per cent of the total nitrogen present in the amino form. The average of these, 71.2 per cent, is the figure recorded in Table V. The extremes in this respect were: Beef sample 11 with 71.6, 74.6, and 75.8 per cent, a difference of 4.2 per cent between the highest and lowest figures, and beef sample 12 with 74.2, 74.4, and 74.7 per cent, the difference being 0.5 per cent. When the difference of 2 per cent was obtained with the first duplicates it was believed to be due to error in procedure. Accordingly, the determinations of the next sample, veal sample 9, were made with the greatest care but with no closer results. Numerous modifications of the method were tried without the desired result. A large number of results were obtained on veal sample 9 and skim-milk sample 2, all of which were low by several per cent and have been omitted from Tables V and XII. The fact that any deviation from the procedure used for beef and veal samples 8 gave uniformly low results led to its use without modification throughout the remainder of the work.

TABLE V.—*Distribution of nitrogen in beef and veal hydrolyzed by hydrochloric acid*

[Total nitrogen=100 per cent.]

Sample No.	Ammonia nitrogen.	Amino nitrogen.	Nonamino nitrogen, by difference.	Melanin nitrogen.	Experiment No.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
Beef 8	7.6	70.9	20.0	1.5	26
Veal 8	7.4	71.2	19.7	1.7	
Beef 10	7.1	74.5	17.6	.8	30
Veal 10	7.1	73.1	19.0	1.0	
Beef 11	6.8	74.0	19.4	.8	32
Veal 11	7.4	70.8	20.5	1.3	
Beef 12	7.5	74.4	17.6	.5	34
Veal 12	5.6	73.5	20.3	.6	
Average:					
Beef 8 to 12	7.2	73.4	18.6	.9	.....
Veal 8 to 12	6.9	72.1	19.9	1.1	.....
Beef 1.1	6.8	75.0	17.4	.8	33
Veal 1.1	6.7	75.1	17.1	1.1	
Casein 1	10.4	71.8	16.1	1.7	29
Casein, by Van Slyke	10.1	72.1	16.1	1.8	.....

CONTROL DETERMINATIONS.—Control determinations on leucin and casein and later on tyrosin were made for the purpose of ascertaining whether errors in procedure were responsible for the unexpected differences between duplicates or whether the nature of the experimental material was such that interfering reactions made it practically impossible to obtain as close duplicates on so complex a mixture as hydrolyzed meat as can be obtained on certain pure amino acids. It is almost certain that the hydrolyzed meat contains a large variety of amino acids, some of which react quantitatively in five minutes; others require several hours; and no particular reaction time is favorable for all taken together. On this point the following quotations from the work of Van Slyke (1911) are of interest:

Time required for different classes of amino derivatives to react quantitatively. Amino groups in the α-position to carboxyl, as in the natural amino-acids, react quantitatively in 5 minutes at 20°. The group in *lysin* requires one-half hour to react completely, lysin being the only natural amino-acid which requires more than 5 minutes. *Ammonia and methylamine* require 1.5–2 hours to react quantitatively. *Urea* requires 8 hours. . . . Amino groups in *purines* and *pyrimidines* require 2–5 hours at 20° (p. 191).

Amino-acids which react abnormally with nitrous acid. Glycocoll and glycyl peptids. Glycyl-glycin, unlike the other peptids, reacts not only with its free primary amino nitrogen, but also as Fischer and Koelker have shown, with a part of the secondary nitrogen in the peptid linking. This is doubtless connected with the peculiar behavior of glycocoll itself when treated with nitrous acid. It gives off not only nitrogen, but carbon dioxide and traces of some other gas, which is not absorbed by permanganate, indicating that decompositions deeper than the deamination occur. The behavior of glycocoll and glycyl peptides can be explained in three ways: . . . (p. 197) The gas measured is about 103 per cent of the theoretical volume of nitrogen . . . (p. 199).

In the determinations on hydrolyzed meat it was observed that almost invariably the nitrogen gas measured would diminish a few tenths of a cubic centimeter in volume, if the gas were passed back into the alkaline permanganate pipette and allowed to remain there overnight. Whether this was due to the glycocoll resulting from the hydrolysis of the different proteins in the meat or to other disturbing factors can not be stated. It is probable that the secondary reactions mentioned above take place when hydrolyzed meat reacts with nitrous acid for 20 minutes, and they contribute to the difficulty of obtaining very close duplicates.

For the control determinations on leucin a sample of Kahlbaum's synthetic leucin was used. This sample was dry and contained 96.4 per cent of the theoretical total nitrogen obtained by the Kjeldahl method, indicating the presence of a non-nitrogenous impurity. Six determinations on *N/10* leucin in 1 per cent (approximately) hydrochloric acid, made at various times throughout the work gave the following results: 95.4, 95.6, 95.3, 95.3, 96.1, and 96 per cent of the theoretical total nitrogen present as amino nitrogen; average, 95.6 per cent. One result, 94.4 per cent, obtained with exhausted permanganate in the absorption pipette,

was omitted from the average. Close duplicates on leucin and on the next control substance, casein, were obtained easily and by the identical methods that failed to produce as close duplicates on hydrolyzed meat.

The casein (casein sample 1) hydrolyzed for the control determinations was prepared in the laboratory in the usual manner, from separator skim milk. The dry protein contained 14.87 per cent of nitrogen and 0.10 per cent of ash. Although small amounts of impurities were probably present in this preparation, it compared favorably with those used by other workers. The hydrolysis of the casein, distillation of ammonia, and determination of amino nitrogen were carried out exactly as with hydrolyzed meat, except that 5 gm. of the dry casein were used instead of 25 gm. of meat. The following results were obtained: 71.37, 72.81 per cent (boiled for 24 hours), and 71.37, 71.73 per cent (boiled for 48 hours). The average of these four, 71.8 per cent, given in Table V, is very close to the figure (72.1 per cent) obtained by Van Slyke (1912, p. 297) and other investigators. The various determinations made with casein sample 1 indicate that the methods used were essentially correct and would yield close duplicates on materials to which they were applicable.

It was thought possible that the fats or their hydrolytic products might interfere with the amino-nitrogen determination, and for this reason determinations were made on beef and veal samples 1.1. These were dry, almost fat-free meat powders, prepared early in the work from beef and veal samples 1 by treating the hashed meats with alcohol and ether (see p. 685). The hydrolysis and determinations were made on these materials as usual, but no better duplicates were obtained. The figures for beef sample 1.1 were 74.1, 75.3, and 75.7 per cent; average, 75 per cent; for veal sample 1.1, 74.1, 74.1, 75.7, and 76.3 per cent; average, 75.1 per cent. The difference between the highest and the lowest figure for veal sample 1.1, 2.2 per cent, corresponds to a difference of 0.6 c. c. of nitrogen gas under the conditions of the determinations, in which the volume of gas actually measured was about 20 c. c.

A sample of tyrosin labeled "Tyrosin, pure, synthetic, Schuchardt" was also used for control determinations. It contained 1.66 per cent of moisture. Calculated to the dry basis the total nitrogen content by the Kjeldahl method was 93.5 per cent of the theoretical. The figures for amino nitrogen were 95.6, 96.0, and 95.3 per cent of the theoretical total; average, 95.6 per cent. In the first determination the gas after being measured was passed back into the absorption pipette, where it remained overnight. As usual, there was a slight diminution in volume—from 95.6 to 95.3 per cent.

It is believed that close duplicates on beef and veal have not been obtained, for reasons inherent in the material; the method used gave good results on comparatively pure leucin, casein, and tyrosin. The comparison between the amino-nitrogen content of beef and that of veal having been made under similar conditions, the data in Table V, although

possibly erroneous to the extent of 1 or 2 per cent, indicate that the differences between the mature beef and the immature veal are too slight to be significant.

NONAMINO NITROGEN.—"The difference between the Kjeldahl and NH_2 determinations gives the nonamino (NH) nitrogen. This includes one NH_2 group, that of the guanidine nucleus of arginine, which does not react with nitrous acid . . ." (Van Slyke, 1912, p. 296).

PERCENTAGE OF WATER IN BEEF AND VEAL

Two 3-gm. portions of each meat, contained in porcelain crucibles, were dried in a vacuum desiccator and two similar portions in a hot-water jacketed oven.

DRYING IN THE HOT-WATER JACKETED OVEN.—The temperature of the interior of the oven ranged from 93° to 95° C. in winter to 95° to 97° C. in summer. The samples were transferred to the oven immediately after being weighed and were dried for 12 hours. A slow stream of clean dry air was passed through the drying chamber for several hours during the drying period, after which the crucibles were transferred to a desiccator, cooled, and weighed.

DRYING IN THE VACUUM DESICCATOR.—The samples of hashed beef and veal were transferred to a Hempel's desiccator; this was evacuated to about 85 mm. of mercury and the drying allowed to take place for two weeks at room temperature. During this time the sulphuric acid was changed once, and the desiccator was evacuated several times.

The dried samples, after being weighed, were transferred at convenient times to Kjeldahl flasks for nitrogen determinations except beef and veal samples 1 and 2. On these ash was determined by igniting the dried material. The results were: Beef sample 1, 1.16 per cent; beef sample 2, 1.10 per cent; veal sample 1, 1.14 per cent; veal sample 2, 1.14 per cent of ash. Calf 1 was 5 days old when killed; calf 2, 3 days. The ages of the others have been given in Table I. The results for water in beef and veal are summarized in Table VI.

TABLE VI.—*Percentage of water in beef and veal*

Sample No.	Dried in vacuum desiccator 2 weeks at room temperature.		Dried in water-jacketed oven 12 hours at 95± 2° C.	
	Beef.	Veal.	Beef.	Veal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	73.25	76.83	73.58	77.10
2	73.59	78.74	74.03	79.83
3	72.98	77.13	71.49	76.37
4	75.28	77.39	74.84	77.39
5	76.46	74.38	75.01	75.79
6	72.69	76.12	73.63	77.60
7	75.13	78.01	75.12	77.98
8	72.54	77.76	71.35	77.93
10	74.48	77.42	75.45	78.38
11	75.11	76.86	75.99	77.05
12	74.43	77.20	74.66	77.54
Average of 22 determinations	74.18	77.08	74.10	77.54

Although care was taken to insure as uniform sampling as possible, the differences between duplicates varied from 0 per cent to 4.70 per cent—i. e., the figures for veal sample 11 were 77.05 and 77.05 per cent; for beef sample 8, 69 and 73.70 per cent. In every case the average of the dulpicates is given in the table. The average of the 44 differences (there were 44 duplicates) was 0.92 per cent. Although theoretically simple, the determination of water in such material as meat is practically very difficult.[1] The results for beef and for veal are strictly comparable in so far as both sets were obtained under the same conditions, but they are not exact in the absolute sense. Had the samples been heated for more than 12 hours in the hot-water oven, the "moisture content" would have been higher, partly because more water would be driven off, and partly because other substances would volatilize, decompositions would begin, etc. Apparently, under the conditions of the determinations, errors which result from heating meat over 100° C. for long periods of time were obviated (Davis and Emmett, 1914).

The figures in Table VI for beef are similar to those obtained by other workers. Richardson and Scherubel (1908, p. 1527, 1552) found an average of 76.35 per cent of moisture in beef which had been dried to constant weight at 100° to 105° C. Grindley and Emmett (1905, p. 659) found 75.46 per cent of moisture in beef dried in a hot-water oven for a length of time not stated.

Obviously, the claim that immature veal ("bob veal") is more watery than beef finds little support in the data obtained, because the difference between the averages, about 3 per cent, is physiologically of no importance.

COMPARATIVE DIGESTIBILITY OF MATURE BEEF AND IMMATURE VEAL IN VITRO

In the following comparative measurements of the speed of proteolysis of beef and veal, an attempt was made to ascertain whether immature veal is more resistant to pepsin and trypsin than beef, as sometimes stated. Three separate methods were used, each of which has its advantages, disadvantages, and errors. In the first method the undigested meat was filtered at the end of the digestion period, dried, and weighed. In the second, nitrogen was estimated in portions of the digestive fluid from time to time, thereby giving an indication of the rate at which nitrogenous substances were going into solution. In the third, the rate of formation of amino nitrogen was estimated in portions of the digestive fluid, indicating the rate at which the amino-nitrogen groups interlocked in the polypeptids present were opened or separated by the trypsin and alkali.[2]

[1] For a discussion of the errors entering into this determination, see Benedict and Manning (1905).

[2] For discussions of the earlier work on artificial digestion, see Grindley, Mojonnier, and Porter (1907, p. 61), and Berg (1909).

SOLUTIONS.—Digestions were made in 0.2 per cent hydrochloric-acid and in 0.5 per cent sodium-carbonate solutions.

ENZYM PREPARATIONS.—The following preparations, all in powder form, were used:

Pepsin 1: A 100-gm. bottle of pepsin (1:3,000), Parke, Davis & Co.; purchased about May, 1912.

Pancreatin 1: A 1-ounce bottle of pancreatin (Parke, Davis & Co.); an old preparation.

Trypsin 1: A 1-ounce bottle of trypsin (Merck); purchased in September, 1912.

Trypsin 2: A 200-gm. bottle of trypsin sicc. (Greubler); imported about March, 1912.

Trypsin 3: A 50-gm. bottle of trypsin (Merck); purchased in August, 1913.

In every case the unopened bottle of enzym preparation was used. Portions were transferred to weighing bottles and dried for several days in desiccators until the loss in weight was slight. The bottles were then stoppered. The day before being used the enzym preparations were dried in a desiccator and portions weighed as needed.

In order to correct the digestion data for nitrogen introduced in the form of enzym, their nitrogen contents were determined. The results are summarized in Table VII. The methods used were similar to those employed throughout the work.

TABLE VII.—*Quantity of N/5 nitrogen per gram of dry enzym preparation*

Preparation.	Total nitrogen.	Ammonia nitrogen.	Amino nitrogen.
	C. c.	*C. c.*	*C. c.*
Pepsin 1	51.1	.....	.....
Pancreatin 1	40.9	.....	.....
Trypsin 1	47.3	None.	.....
Trypsin 2	70.1	62.5	2.0
Trypsin 3	47.0	1.0	22.8

All of the digestion experiments were begun with freshly hashed beef or immature veal, except experiments 5 to 8, in which powdered meats beef sample 1.1 and veal sample 1.1, were used. These were prepared as follows:

Veal sample 1.1: Seven kgm. of veal sample 1 were hashed and transferred to two 8-liter wide-mouth bottles. Seven liters of 50 per cent alcohol were added and the mixture well stirred. After 24 hours the 50 per cent alcohol was strained off through cheesecloth and replaced with an equal volume of 75 per cent and the next day with 95 per cent alcohol This was followed by two treatments with 2 liters of absolute ether. The ether was removed by straining through cheesecloth and squeezing

the material, after which most of the ether was removed by exposing the veal to the air in large crystallizing dishes. The veal was then heated in the hot-water oven at 85° C. (flame out) for two hours, and bottled.

Beef sample 1.1: Fourteen hundred grams of beef sample 1 were treated with alcohol and ether in exactly the same way as veal sample 1.1, using 1,400 c. c. of alcohol, etc.

When portions of these powdered-meat preparations were weighed for digestions, portions were also weighed for moisture determinations, so that the final weights were based on the dry material. Total nitrogen per gram of beef sample 1.1, 57.2 c. c. *N*/5 nitrogen; per gram of veal sample 1.1, 57.8 c. c. *N*/5 nitrogen. Other analytic data are given in Table V.

FIRST METHOD: WEIGHING THE UNDIGESTED MEAT RESIDUES

Portions of 5 gm. each of the raw hashed beef and veal were weighed into 200 c. c. Erlenmeyer flasks. After adding 40 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for 1 hour. They were then cooled, weighed, and the evaporated water replaced. To each flask 50 c. c. of 0.4 per cent hydrochloric acid were added, followed shortly afterwards by the addition of 10 c. c. of the pepsin solution. Three flasks containing beef and three containing veal were generally used in a single experiment (see Table VIII). In the controls on the acid 10 c. c. of water instead of the pepsin solution were added.

The digestion was considered to have begun when the pepsin was added. During the digestion period the flasks were rotated occasionally, so as to mix the contents. When the digestion period had ended, the filtration of the residue, consisting of undigested meat, fat, etc., was begun. For this purpose loose-textured filter papers (Schleicher & Schull's No. 589, white band, 15 cm.) were used. These papers, contained in weighing bottles, had previously been dried for several hours at 95° C. in the hot-water oven until the change in weight after a second drying was slight. Drying such papers to absolutely constant weight was as difficult as drying meat to constant weight without decomposition or oxidation.

It is at this point that the worker loses control over the method. When filtration was rapid, which sometimes happened, the separation of undigested meat from the pepsin-hydrochloric-acid solution ended the digestion period quite sharply, so far as the residue was concerned. But, as was generally the case, filtration was slow because the residue was gelatinous and clogged the filter, and it was not possible to end the digestion period shortly after filtration was begun because digestion continued as long as the pepsin-hydrochloric-acid solution was in contact with undigested meat. Fortunately, the digestive process becomes slow as the meat approaches complete digestion, so that the error from this source probably amounts to less than 10 per cent of the correct result.

When filtration was complete or nearly so, the residues were washed with water, transferred with the paper to the corresponding weighing bottles, and dried to approximately constant weight at 95° C. in the hot-water oven. From the data for moisture, the original 5-gm. portions of fresh meat were calculated to the dry weight. The weight of the dry, undigested residue divided by the corresponding weight of dry meat gave the percentage of beef or veal present as undigested residue (see "Percentages of meat digested," p. 700).

ACID PROTEINATE.—The value of the determination of acid proteinate in digestion mixtures has been pointed out by Gies (Hawk and Gies, 1902). The first step in the

digestion of a protein by pepsin-hydrochloricacid solution is the combination of the protein and the acid to form a class of substances known as acid proteinates. These are soluble in dilute acids and alkalies, but are insoluble in water.

The filtrates obtained at the end of the digestion period contained (1) the acid proteinates and (2) the next cleavage products of the acid proteinate, the proteoses and peptones. A measured amount of filtrate, generally between 50 and 80 c. c., taken before the washing of the residue was begun, was nearly neutralized with *N*/5 sodium hydroxid. The exact amount added varied in the different experiments; calculated to 100 c. c. of filtrate it varied around 21 c. c. The 100 c. c. of 0.2 per cent hydrochloric acid in which the digestions were made were equivalent to 28 c. c. of approximately *N*/5 sodium hydroxid. The addition of alkali was stopped when a flocculent precipitate of acid proteinate was thrown down. The mixture was then rapidly brought to a boil and filtered on a weighed paper. This was dried along with the undigested residues, and the results calculated in the same way.

The difficulties involved in promptly checking the action of the pepsin at the end of the digestion period were very apparent to Grindley, Mojonnier, and Porter (1907, p. 68), who after many trials found that the addition of formaldehyde solution to a digestion mixture brought the digestion to a close. Differences in length of time required for filtration will not then involve the error previously mentioned. This method, however, is not the only one. By using small amounts of pepsin the digestion period may be made long; and then it makes little difference whether a particular mixture requires a few more or a few less hours to filter completely. An objection to this procedure is that the acid alone in the control may digest as much as the acid plus the small amount of pepsin, and the action of the pepsin under such conditions can not be measured with certainty. Further, the amount of pepsin must not be large enough to permit the digestive processes to go to completion, for the undigested residue then obtained represents material not digestible under the conditions, and no information is obtained regarding the rate at which digestion took place. If allowed time enough, both a fast horse and a slow horse will be found at the same place at the end of a race. In experiment 13, Table VIII, the undigested residues obtained after long digestion with fairly large amounts of pepsin represented the amount of meat constituents not digestible by the pepsin-hydrochloric-acid solution.

No information as to whether the beef or the veal digested faster could be obtained from such data. That the residues in this experiment were almost certainly fat is indicated by the results of Table IX, with which experiment 13 is comparable because the concentration of pepsin was the same in both—i. e., 10 mgm. to 100 c. c. of 0.2 per cent hydrochloric acid. Under these conditions practically all of the nitrogen in the beef and veal went into solution in 24 hours, leaving the fat, which is not digested by pepsin-hydrochloric-acid solution. Fat determinations were not made. According to Fish (1911, p. 132), beef contains more fat than ordinary veal. This is probably still more true of immature veal. The larger residues from beef in experiment 13 are in accord with the data of Fish.

TABLE VIII.—*Comparative digestibility of mature beef and immature veal in pepsin-hydrochloric-acid solution*

Experiment No.	Digestion period.	Filtrate neutralized after—	Pepsin 1.	Percentage of beef present as—		Percentage of veal present as—		Temperature.
				Undigested residue.	Acid proteinate.	Undigested residue.	Acid proteinate.	
			Mgm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	°C.
BEEF AND VEAL SAMPLES 1								
1	8 days	4 hours	.....	79	4	74	5	Room.
			.....	79	8	73	5	
			0.01	64	16	63	9	
			.01	63	12	60	10	
2	8 days	4 hours	.10	22	31	29	21	Do.
			.10	25	29	26	22	
3	1½ hours	½ hour	.....	84	4	85	4	
			10.0	43	(a)	52	5	40
			10.0	43	11	54	6	
4[b]	3 hours	½ hour	.....	66	22	45	12	
			10.0	30	14	34	7	40
			10.0	24	16	27	8	
BEEF AND VEAL SAMPLES 1.1								
5[c]	46 days	24 hours	.....	12	11	20	16	Room.
			.01	11	37	37	4	
			.01	8	23	43	34	
6	10 days	10 hours	.....	82	11	78	10	
			.10	15	38	42	34	Do.
			.10	17	38	34	35	
7	4 hours	1 hour	.....	88	10	94	6	
			10.0	11	25	33	21	Do.
			10.0	11	26	33	21	
8	3 hours		.....	87		85		
			10.0	25		27		40
			10.0	25		28		
BEEF AND VEAL SAMPLES 2								
9	4 hours	½ hour	.....	84	6	79	4	
			10.0	27	7	21	7	40
			10.0	30	7	21	6	
10	4 hours		.....	78		73		
			10.0	42		32		40
			10.0	44		29		
11	4 hours	½ hour	.....	76	11	77	3	
			10.0	36	9	25	6	40
			10.0	44	9	28	6	
12	4 hours		.....	80		77		
			10.0	53		32		40
			10.0	50		33		
13	23 days	4 hours	.....	74	14	73	9	
			10.0	20	0	15	0	
			10.0	15	0	12	0	Room.
			10.0	20	0	12	0	
			10.0	23	0	11	0	
			10.0	19	0	.....	.....	

[a] Determination lost.

[b] The flasks containing the 5-gm. portions of hashed beef and veal were kept in cold storage at 2° C. for three weeks, during which time autolysis went on. This probably accounts for the small residues in the blanks, which contained hydrochloric acid but no pepsin. While in cold storage the flasks contained nothing but the meat.

[c] Experiment 5 is to be rejected. The continued action of molds during the digestion period invalidated the results.

A second method of checking the action of the pepsin-hydrocloric-acid solution used in experiments 8, 10, and 12 involved nothing more than the neutralization of the digestion mixture at the end of the desired time. Pepsin digests in the presence of free acid; it does not act in neutral solutions with any appreciable speed. Thus in experiment 10, and in experiment 12, which was a repetition of experiment 10, exactly four hours after the digestion was begun by adding the pepsin solution to 5-gm. portions of meat suspended in 100 c. c. portions of 0.2 per cent hydrochloric acid, the entire mixture was neutralized by the addition of 21 to 25 c. c. of *N*/5 sodium hydroxid. This checked the peptic action at once, but also precipitated the acid proteinate. The mixture was then quickly brought to a boil, after which filtration, whether fast or slow, may be continued at the convenience of the worker. Obviously the residue in this case does not give as detailed information as that obtained by filtration of undigested residue and precipitation of acid proteinate in the filtrate. In experiments 10 and 12 the veal digested a little faster than the beef.

In experiments 5 to 8, practically fat-free beef and veal, prepared as described on page 685, were used. The object was to eliminate the error due to the fat, which, when present, is weighed with the undigested protein. One-gm. portions of the dry powders were used instead of the 5-gm. portions of fresh meat. Otherwise the procedure was the same as in the other experiments, except that, in so far as the proteins present had already been coagulated by exposure to alcohol, ether, and a temperature of 85° C., heating the mixture of meat powder and water in a boiling-water bath was omitted. The results in experiment 5 were invalidated by molds. In experiments 6 to 8 the results indicate a slightly more rapid digestion of beef sample 1.1.

The most interesting results in Table VIII are those of experiments 1, 2, and 6. In experiment 1 so minute a quantity of pepsin as 0.01 mgm. in 100 c. c. of 0.2 per cent hydrochloric acid exerted an equally distinct digestive action on both the beef and veal. With 0.1 mgm. of pepsin the digestion was unmistakable, indicating that in these particular cases the immature veal was as susceptible to the action of minute amounts of pepsin as the mature beef. To ascertain whether this was true or not was the object of experiments 1 and 2.

It will be noticed that in the experiments summarized in the table the amounts of pepsin used varied from 0.01 mgm. to 1,000 times this amount—i. e., 10.0 mgm. A wide range of enzym concentration in such work is not only desirable but almost necessary. What is true at one concentration of enzym may not be true at another very different one. Thus, Berg and Gies (1907) found that in acetic acid fibrin would digest very slowly when the amount of pepsin present was comparatively small, but in the presence of large amounts of this enzym digestion proceeded with a wholly unexpected speed.

A comparison of the results for beef in Table VIII with some of the data obtained by Grindley, Mojonnier, and Porter (1907, p. 66) in their artificial-digestion experiments can not very well be made. These investigators used 250 mgm. of pepsin per 100 c. c. of 0.33 per cent hydrochloric acid. The kind of pepsin preparation used was not stated, but, assuming it to be the usual 1 to 3,000 product, their digestion mixtures contained 25 times as much pepsin as the strongest digestion mixtures mentioned in Tables VIII or IX. Their conditions of comparatively high pepsin and high acid concentration probably were not favorable for the detection of small differences in digestibility, although these conditions may have been desirable for other reasons.

Perhaps the only work with which the data of Table VIII can be compared are the recent results obtained by Fish (1914) on the comparative digestibility of beef, market veal, and immature veal. In the absence of a statement pertaining to the treatment of the meats, the inference may perhaps be drawn that the digestion experiments were made on raw meats. Otherwise, the general method and conditions of Fish's digestion experiments were similar to those in experiments 1 to 13. Samples from 22 immature

veal calves were compared with an almost equal number of samples of market veal and beef, using "3.35 milligrams of scale pepsin" in 100 c. c. of 0.2 per cent hydrochloric acid. Fish (p. 52–53) concludes this part of the work with the following statement:

> The results show that, as regards the averages, the differences in the digestibility of the tissues of bob veal and market veal are so slight as to be negligible; but such as they are, they are slightly in favor of the bob veal as a whole. The differences between the beef and veal is [sic] more noticeable, but the apparent greater digestibility of the veal may be due in part to the fact that as a rule there is a slightly smaller percentage of water present in the beef as well as a somewhat greater amount of connective tissue. As the greatest difference shown by the averages is but 3 per cent under the conditions of the experiments, it would indicate no serious difficulties in the digestibility of any of the material.

A redeeming feature of the method used in experiments 1 to 13 is its simplicity, both in the technic used and the equipment required. That the results obtained are substantially correct is indicated by the fact that repetitions of the measurements, using different methods, involving different errors, yielded similar results.

SECOND METHOD: MEASURING THE RATE OF FORMATION OF PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN

Into each of two 2-liter Jena Erlenmeyer flasks 100 gm. of freshly hashed beef were weighed to the 0.1 gm. Similar portions of veal were weighed into two similar flasks. After adding 750 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for one hour. They were then cooled, weighed, and the evaporated water replaced. The stoppered flasks remained in cold storage overnight. Two of these, one of beef and one of veal, were used for the determination of extractive nitrogen as already described on p. 673. The next morning the flask containing the beef and the flask containing the veal for the digestion experiment were quickly warmed to 40° C. The dry, powdered enzym was then added, followed by 1 liter of 0.4 per cent hydrochloric acid or of 1 per cent sodium-carbonate solution. Water was then added to bring the final volume up to 2,000 c. c. In this way every digestion experiment was begun with 100 gm. of beef or veal, plus 2,000 c. c. of 0.2 per cent hydrochloric acid when pepsin was used (see Table IX), or 2,000 c. c. of 0.5 per cent sodium carbonate when trypsin was used (see Table X). During the course of the digestion the flasks were kept in a 40° C. water bath, except when they were removed to mix their contents or to take samples for analysis. The treatment of the digestion mixtures containing pepsin-hydrochloric-acid solution and those containing trypsin-sodium carbonate solution will be described separately.

DIGESTION IN PEPSIN-HYDROCHLORIC-ACID SOLUTION.—During the earlier part of the experiment the contents of the flasks were mixed about every 15 minutes. Later, when most of the meat had gone into solution, the mixing was done at longer intervals, but always the same for both flasks. In the experiments summarized in Table IX the rate of digestion was measured at the time intervals there indicated by removing 100 c. c. portions of supernatant digestion fluid and determining in this the amount of nitrogen present as acid proteinate, proteoses, and peptones. By difference the nitrogen in the undigested residue could be obtained. If, for example, it was desired to obtain data on veal for one hour's digestion, the veal mixture was well mixed 45 minutes after the digestion was begun and was allowed to remain in the water bath for 10 minutes, in order to allow meat particles to settle to the bottom of the flask. The flask was then removed from the bath, and with a calibrated 100 c. c. pipette 100 c. c. of the supernatant suspension was transferred to a 200 c. c. Erlenmeyer flask. Exactly 60 minutes after the digestion began, the action of the pepsin-hydrochloric-acid solution was stopped by nearly neutralizing the contents of the 200 c. c. Erlenmeyer flask by the addition of $N/5$ sodium hydroxid and bringing it to a boil by heating directly over a Bunsen burner. The flask containing the digestion mixture was

replaced in the bath. The quantities of *N*/5 sodium hydroxid used varied from 18 to 29 c. c. The neutralization is satisfactory when a flocculent precipitate appears. In the same way 100 c. c. of the digestion fluid from the beef mixture were removed and neutralized 60 minutes after starting the beef digestion.

In this way portions of the digestion mixtures of beef and veal were removed for neutralization on the minute, at intervals of 1, 2, 4, 7, and 24 hours. Fifteen minutes before neutralization the flask contents were mixed and allowed to stand for 10 minutes. A 100 c. c. portion was then removed from the bulk of the digestion mixture 5 minutes before neutralization.

The precipitated acid proteinate was filtered, washed, and nitrogen was determined by the Kjeldahl method. The results obtained are given in Table IX under the heading "Quantity of *N*/5 acid-proteinate nitrogen."

The filtrate was transferred to a Kjeldahl flask and the total nitrogen determined. This filtrate contained nitrogen derived from (1) the proteoses and peptones formed by the digestion of the meat, (2) the extractives present before digestion began, and (3) the pepsin. The figure for total nitrogen obtained on the filtrate is the sum of these three. The data recorded in Table IX under the heading "Quantity of *N*/5 proteose and peptone nitrogen" are the figures actually obtained and corrected for the sum of the extractive and pepsin nitrogen. Thus, in experiment 14 the results obtained for one hour's digestion of beef sample 3 were, for the precipitated acid proteinate, 2.7 c. c. of *N*/5 nitrogen; for the filtrate, 23.8 c. c. From this latter figure there was subtracted 8.0 c. c., this being the sum of the extractive nitrogen in that sample of beef at that time, and the nitrogen present in the pepsin added. The method of determining extractive nitrogen is described on page 673.

During the digestion the water contained in the meat is liberated and dilutes the digestion fluid to a slight extent. No correction for this was made, except in those particular cases where the correction is indicated.

The "theoretical maximum" for proteose and peptone nitrogen in 100 c. c. of digestion fluid was calculated in the following manner: The sum of the total nitrogen in 100 gm. of fresh meat plus the pepsin nitrogen was divided by the volume of the digestion fluid at complete digestion—i. e., 2,000 c. c. plus the volume of water in the 100 gm. of meat.

By the term "Age of meat, days," at the bottom of Table IX is meant the number of days the meat was in cold storage before being boiled. Thus, in experiment 21 beef sample 6 and veal sample 6 were hashed and boiled after 13 days in cold storage, and on the next day digestion was begun. These figures do not refer to the age of the calf when killed, this having been given in Table I.

It will be noticed that the theoretical maximum for proteose and peptone nitrogen is approximately 50 c. c. of *N*/5 nitrogen in nearly all the experiments. In order to obtain the percentage of nitrogen present as proteoses and peptones at any time, it is only necessary to multiply the corresponding figure by 2. Thus, in experiment 19, at the end of seven hours approximately 82 per cent of the veal (41.0÷48.0) had been transformed into proteoses and peptones. It is obvious that both the beef and the veal were digested with practically the same speed and that at the end of 24 hours the transformation into proteoses and peptones was complete.

For practical purposes the digestive process may here be regarded as taking place in two stages: (1) The transformation of the native meat proteins to acid proteinate by combination with the hydrochloric acid, and (2) the cleavage of the acid proteinate into the smaller molecules of proteoses and peptones.

The data in Table IX indicate that both processes took place with equal speed in the beef and veal.

The undigested residues weighed in experiment 13, Table VIII, probably contained very little nitrogen. The concentration of pepsin in experiments 9 to 13 was the same

as in the experiments in Table IX. By comparing the results of experiment 13 with those of experiment 14, for example, it will be apparent that the undigested residues in experiment 13 give an imperfect idea of the amount of indigestible protein present in beef and veal; according to the data of Table IX practically all of the nitrogen was in soluble form at the end of 24 hours.

The conditions of the experiments in Table IX were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.2 per cent hydrochloric acid plus 200 mgm. of pepsin 1. For nitrogen determinations 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE IX.—*Rate of formation of proteoses and peptones in pepsin hydrochloric-acid solution*

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 PROTEOSE AND PEPTONE NITROGEN

Digestion period.	Experiment No. —									
	14		17		19		21		23	
	Beef sample 3.	Veal sample 3.	Beef sample 5.	Veal sample 5.	Skim milk sample 1.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
Hours.										
1	15.8	10.3	15.6	16.8	27.7	12.6	15.7	12.1	17.1	11.6
2	24.7	20.5	26.8	26.6	37.2	23.4	23.4	19.3	27.9	18.5
4	33.8	31.1	37.8	36.3	43.8	34.6	33.1	29.6	37.9	29.0
7	41.8	39.5	45.3	41.4	46.2	41.0	41.0	37.8	44.2	36.7
24	50.7	47.1	52.3	46.7	50.4	46.6	52.8	(a)	52.9	45.5
Theoretical maximum	51.8	48.3	53.1	48.0	54.8	48.0	53.8	43.8	54.2	47.3
Extractive nitrogen	7.5	8.9	7.2	7.8	3.7	7.8	8.1	7.7	7.5	11.1
Pepsin nitrogen	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 ACID PROTEINATE NITROGEN

Hours.										
1	2.7	1.5	4.6	4.2	26.0	2.4	2.8	1.6	3.3	1.5
2	3.9	2.8	4.8	4.9	17.3	4.5	4.7	3.2	5.2	3.7
4	3.4	3.6	5.0	4.6	10.8	4.6	5.4	4.3	4.9	4.0
7	3.3	4.3	3.8	5.1	8.2	5.6	5.2	5.9	4.1	4.2
24	3.0	4.0	3.3	4.2	4.3	4.9	3.7	4.5	2.7	4.0
Age of meat, days	8	8	0	0	18	18	13	13	3	3

(a) Determination lost. Result obtained at 53 hours (47.1 c. c.) is probably incorrect, being larger than the theoretical maximum for that mixture.

The results of the experiments in Table IX can be plotted, and curves, of which the following are typical, obtained (fig. 1).

After several comparisons of veal with beef showed no appreciable differences between the two as regards their behavior in pepsin hydrochloric acid or in trypsin sodium carbonate, it was desirable to compare the veal with some other protein material in order to be certain that the method used would detect a difference in the

rate of digestion when such a difference existed. Accordingly, in experiment 19, veal sample 5 was compared with a sample of raw skim milk obtained in the fresh condition from the Dairy Division, Bureau of Animal Industry. Instead of 100 gm. of beef, 600 gm. of the skim milk were transferred to a 2-liter Erlenmeyer flask. The specific gravity of skim-milk sample 1 was 1.0352 at 26° C., and, hence, the volume of the 600 gm. was 600/1.0352, or 579.2 c. c. This was regarded as if it were 100 gm. of beef plus 479 c. c. of water. To this amount, 316 c. c. of water were added, the milk being kept in a boiling-water bath for five minutes. It was kept in cold storage overnight with veal sample 5; the next morning it was treated in the usual way along with this sample. At the beginning of the digestion the volume of the skim-milk digestion mixture was 2,096 c. c., which is practically the volume of the meat mixtures—i. e., 2,000 c. c. plus the volume of 100 gm. of meat, which lies between 75 and 100 c. c. A similar sample of skim milk in 0.2 per cent hydrochloric acid was used for the determination of extractive nitrogen. Skim-milk sample 1 con-

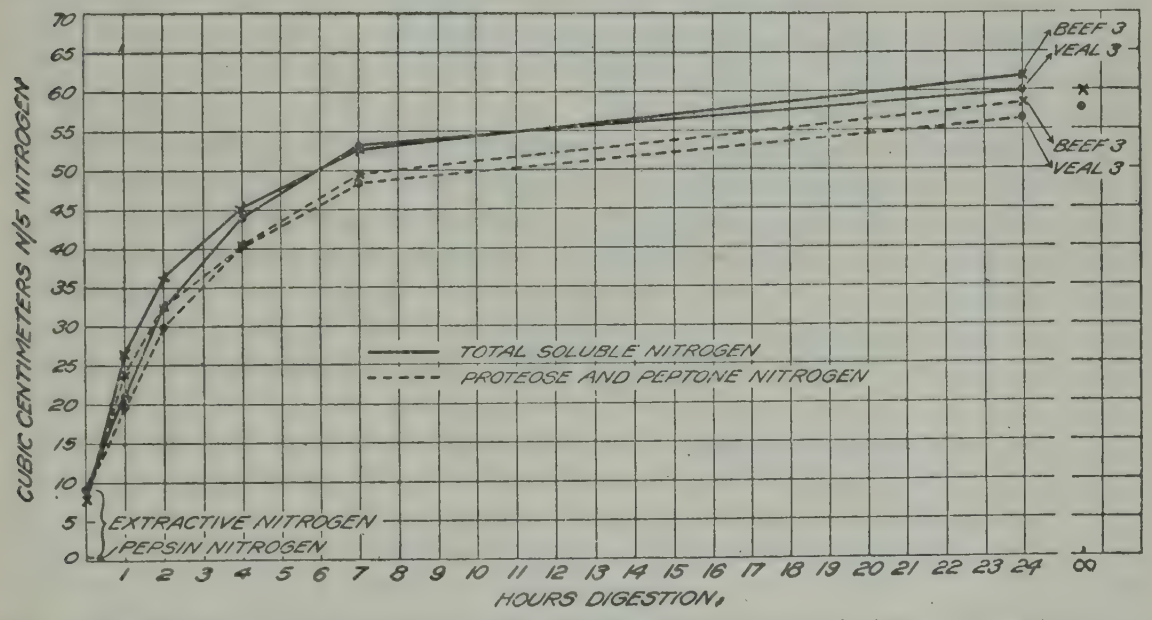

FIG. 1.—Experiment 14. Curve showing the quantity (in cubic centimeters) of $N/5$ nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat; used 100 gm. of meat, 2,000 c. c. of 0.2 per cent hydrochloric acid, and 200 mgm. of pepsin 1.

tained 2.05 c. c. of $N/5$ nitrogen per gram, or 0.574 per cent. The extractive nitrogen was 6.3 per cent of the total nitrogen.

In precipitating the undigested proteins and the acid proteinate by neutralization and heat, care was taken to test the filtrates with acid and alkali, in order to be certain that precipitable protein was not present in any of the filtrates. The complete precipitation, though troublesome, was not difficult. The precipitates, containing both undigested proteins and acid proteinate, were determined for nitrogen by the Kjeldahl method in the usual manner and the results recorded under the heading "Quantity (in cubic centimeters) of $N/5$ acid proteinate nitrogen." The figures for proteose and peptone nitrogen obtained from the filtrates indicate that this transformation was more rapid in the skim milk than in the veal. This is, of course, easily accounted for by the fact that the skim-milk proteins were in solution or suspension at the beginning of the digestion, while the veal particles took time to go into solution.

DIGESTION IN TRYPSIN SODIUM CARBONATE SOLUTION.—In general, these experiments were carried out in exactly the same way as the digestions in pepsin hydrochloric acid solution. Dry, powdered trypsin preparations were used. Portions of these were weighed and transferred to the digestion mixtures in the same way as the pepsin. Instead of 1 liter of 0.4 per cent hydrochloric acid, the same volume of 1 per cent sodium carbonate was added. The digestions in experiments 15 to 34 (Tables X and XI)

were all made in 0.5 per cent sodium carbonate. Although trypsin 1 and trypsin 3 had the same total nitrogen contents (see Table VII), trypsin 1 was the more active preparation. This is evident from the fact that in experiments 18, 20, and 22 (Table X) digestion had proceeded as far in seven hours as in experiments 32 and 34 at the end of six hours, although in the latter experiments twice the weight of trypsin was used.

The 100 c. c. portions of digestion fluid were neutralized with 24.5 c. c. of 2 N 2/5 sulphuric acid, the exact strength of which was N 2/5 × 0.98. This was sufficient to neutralize the sodium carbonate present and leave about 0.5 c. c. of the acid in excess, preventing the escape of ammonia when the mixture was brought to a boil. The filtration and determination of total nitrogen in the precipitated alkali proteinate and in the filtrate were carried out as described in the acid digestions.

It is to be noted that, while small amounts of pepsin in hydrochloric acid will rapidly digest meat proteins to the proteose and peptone stage but no further, trypsin, although much slower in its action, will further split the meat proteins into amino acids. This is the reason for the data under "Quantity of $N/5$ proteose, peptone, and amino-acid nitrogen" in Tables X and XI. The statement of results in Table X is, in general, similar to that in Table IX.

The conditions of experiments 15 to 24 were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium-carbonate solution plus 2.000 gm. of trypsin 1; except experiment 15, in which 2,000 gm. of pancreatin 1 was used. For nitrogen determinations, 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE X.—*Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution*

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN

Digestion period.	Experiment No.											
	15		16		18		20		22		24	
	Beef sample 4.	Veal sample 4.	Beef sample 4.	Veal sample 4.	Beef sample 5.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
Hours.												
1	5.0	6.5	9.3	7.7	11.4	9.3	9.2	8.4	7.7	6.2	7.8	8.1
2	10.7	11.7	16.9	15.4	20.7	16.6	18.5	16.0	14.5	13.4	13.8	14.0
4	16.5	17.9	[a]28.3	[a]28.1	29.8	25.6	29.1	24.6	23.0	21.1	20.3	19.2
7	22.2	23.5	[a]34.1	[a]34.7	38.7	33.3	37.4	31.5	30.2	26.2	26.1	22.9
24	33.7	34.1	42.9	43.6	47.6	42.2	46.7	40.6	43.7	36.8	36.9	28.5
Theoretical maximum	52.7	46.1	53.2	46.6	53.4	48.3	54.1	44.1	54.1	44.1	50.1	32.5
Extractive nitrogen	7.2	8.4	7.2	8.4	7.2	7.8	8.1	7.7	8.1	7.7	11.9	26.2
Trypsin nitrogen	4.1	4.1	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 ALKALI-PROTEINATE NITROGEN

1	3.7	2.9	3.7	2.0	3.3	2.9	4.0	2.6	3.0	2.3	3.1	3.9
2	6.8	4.2	6.8	5.3	4.2	4.4	4.8	4.0	4.0	6.5	4.7	3.0
4	7.6	5.1	[a]6.2	[a]5.3	4.2	5.7	5.0	5.0	4.3	9.8	5.0	3.3
7	8.6	6.9	[a]5.7	[a]8.2	4.4	7.6	5.5	5.8	4.8	10.6	4.9	2.0
24	6.9	8.3	3.3	4.2	4.3	4.9	3.1	5.1	3.0	6.7	3.6	1.6
Trypsin nitrogen	.....	.....	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Age of meat, days	1	1	9	9	7	7	2	2	21	21	33	33

[a] Results are for five and eight instead of four and seven hours.

In experiments 15 to 24, because of the comparatively large weight of trypsin used, it was desirable to ascertain how much of the trypsin nitrogen appeared in the neutralized digestion filtrate and in the precipitate of alkali proteinate, in order that both may be corrected by the amounts found. Accordingly, two portions of trypsin 1, each weighing 100 mgm., were dissolved in 100 c. c. of 0.5 per cent sodium carbonate and precipitated with 48 c. c. of *N*/5 sulphuric acid, as in the digestion experiments. The mixtures were heated to a boil and filtered. The total nitrogen (*N*/5) in the filtrates was 4.25 and 4.40 c. c.; in the precipitates, 0.47 and 0.70 c. c. The averages of these are recorded in Table X, and both were used as corrections, as already described on page 691. For trypsins 2 and 3 the term "trypsin nitrogen" in Table XI means the total nitrogen in the trypsin present in the 100 c. c. of digestion fluid. Trypsin 2 contained approximately 90 per cent of its nitrogen as ammonia, and consequently the amount precipitated with the alkali proteinate was disregarded. The results for alkali proteinate in experiments 31 to 34 with trypsin 3 showed that the correction for alkali proteinate derived from the trypsin must have been similar to that in trypsin 1, and the determination of this correction was omitted.

In experiment 18, for example, 100 c. c. of veal sample 5 digestion fluid were neutralized exactly four hours after the digestion began, and the mixture was brought to a boil and filtered. The filtrate contained 37.7 c. c. of *N*/5 nitrogen, of which 4.3 c. c. were derived from the trypsin present and 7.8 c. c. from the extractives present before the digestion was begun; and the figure recorded, 25.6 c. c., is the amount of proteose, peptone, and amino-acid nitrogen actually formed by the digestive process. The precipitated alkali proteinate contained 6.3 c. c. of *N*/5 nitrogen, of which 0.6 c. c. was derived from the trypsin. The corrected figure, 5.7 c. c., is recorded in Table X.

The results with trypsin are practically the same as those with pepsin. They indicate that both the beef and the veal digested with practically the same speed. The presence of only small amounts of alkali proteinate through the experiments indicates that just as soon as the beef or the veal goes into solution as alkali proteinate this is promptly split into the simpler molecules of proteoses, etc.—i. e., the equality in speed of digestion pertains both to the first and to the later stages in the digestive process for both beef and veal. At no time was there any indication that either the beef or the veal contained any nitrogenous substances resistant to the action of the trypsin. In experiments 16 to 24, Table X, approximately 90 per cent of the veal had gone into solution at the end of 24 hours, with similar results for the beef.

In experiments 26 to 34, Table XI, the rate of digestion was measured by both the second and third methods. The comparisons between veal sample 9 and skim-milk sample 2 in experiments 27 and 28 were made for the purpose of ascertaining whether the method used would detect a difference in rate of digestion when such a difference was large. Experiment 28 was a repetition of experiment 27. On account of the comparatively vigorous action of pepsin-hydrochloric-acid solution veal sample 5 in experiment 19 very soon "caught up" with skim-milk sample 1; but in experiments 27 and 28 the striking difference between the rate of digestion of skim-milk sample 2 and veal sample 9 was brought out by the less vigorous cleavage of the trypsin-sodium-carbonate solution. The treatment of skim-milk sample 2 was similar to that of skim-milk sample 1. Skim-milk sample 2 was obtained by skimming, with the aid of a siphon, a sample of ordinary pasteurized milk obtained from a dealer. One gm. of skim-milk sample 2 contained 1.88 c. c. of *N*/5 total nitrogen, or 0.529 per cent. The extractive nitrogen in experiments 27 and 28 was 11.6 and 14.2 per cent, respectively, of the total. The specific gravity was 1.0334 at 26° C. Six hundred gm. of skim-milk sample 2 were weighed into a 2-liter Erlenmeyer flask. The calculated volume of the skim milk was 580.4 c. c. To this 316.4

gm. of water were added and the flask kept in a boiling-water bath for 15 minutes. The temperature inside the flask was 89° C. The evaporated weight of water was replaced. The heated skim milk was kept overnight in cold storage and digested the next morning with veal sample 9, after the addition of 2,000 gms. of trypsin 2, 1 liter of 1 per cent sodium carbonate, and 200 c. c. of water; total volume, 2,098 c. c. The neutralization of the 100 c. c. portions of digestion fluid were made, as usual, with 24.5 c. c. of *N*/0.4 sulphuric acid, followed by heating to a boil. Extractive nitrogen was determined in a similar portion of skim-milk sample 2 in 0.5 per cent sodium carbonate; 27.5 and 29 c. c. of *N*/0.4 sulphuric acid were used for the pre-

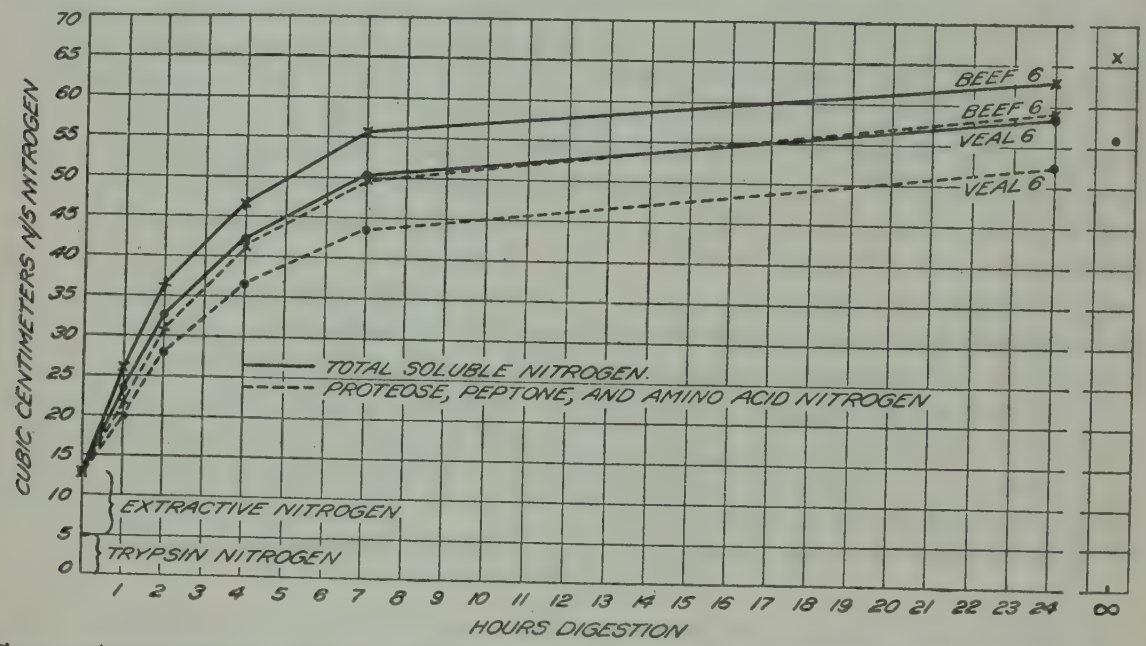

FIG. 2.—Experiment 20. Curve showing the quantity (in cubic centimeters) of *N*/5 nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat; used 100 gm. of meat, 2,000 c. c. of 0.5 per cent sodium carbonate, and 2,000 gm. of trypsin 1.

cipitation of 30 gms. of skim-milk sample 2 contained in 100 c. c. of 0.5 per cent sodium carbonate.

In the two following diagrams (figs. 2 and 3) the data of experiments 20 and 28 are graphically represented.

THIRD METHOD: MEASURING THE RATE OF LIBERATION OF FREE AMINO GROUPS

These determinations were made on the same digestion mixtures used in experiments 26 to 34. Portions of 100 c. c. of the supernatant digestion fluid were removed for the determination of the nitrogen present as alkali proteinate, proteoses, peptones, etc., as already described. In addition, 10 c. c. portions of the digestion fluid were transferred to the Van Slyke amino-nitrogen apparatus, and free amino nitrogen was determined by the method already described on p. 680.

In this method, as in the previous ones, particular care was taken to check the action of the trypsin on the minute. The digestion mixtures in the 40° C. water bath were mixed 15 minutes before the time intended for the determination. The undigested meat particles were allowed to settle for 10 minutes. During this time the amino-nitrogen apparatus was made ready for the determination. Two or three minutes before the digestion period was to be brought to a close, 10 c. c. of the supernatant digestion fluid were transferred to the apparatus, and exactly at the expiration of the digestion period the digestion fluid was allowed to enter the reaction chamber of the apparatus. This brought the digestion to a close.

The results obtained are summarized in Table XII. Amino nitrogen in the extractives was determined in portions of the same filtrates that were used for total extractive-

nitrogen determinations (see p. 696). The results for amino nitrogen in trypsin 2 were obtained by introducing a solution of the enzym in 0.5 per cent sodium carbonate directly into the amino-nitrogen apparatus. The ammonia nitrogen present in this preparation reacted completely with nitrous acid in the 20 minutes' reaction period used uniformly in the determinations. The small amount of ammonia nitrogen present in trypsin 3 permitted the determination of amino nitrogen in the residue obtained after ammonia removal and the use of this figure as the correction (see Table V II).

The conditions of experiments 26 to 34 were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium-

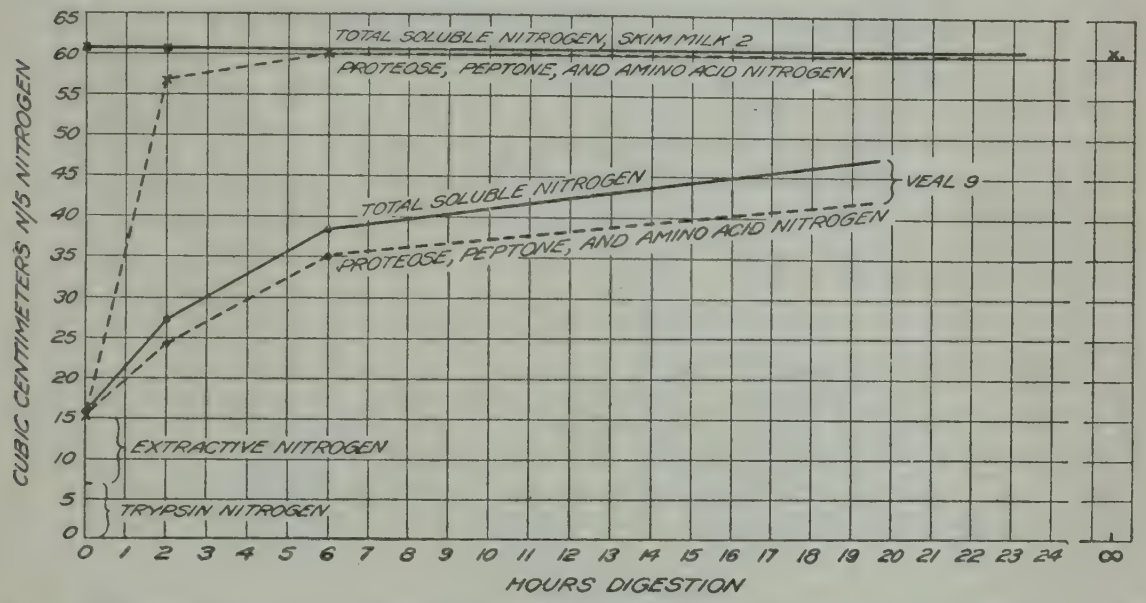

FIG. 3.—Experiment 28. Curve showing the quantity (in cubic centimeters) of $N/5$ nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat or 30 gm. of skim milk; used 100 gm. of veal sample 9 or 600 gm. of skim-milk sample 2, 2,000 c. c. of 0.5 per cent sodium carbonate, and 2,000 gm. of trypsin 2.

carbonate solution plus the amount of trypsin indicated in Table XII. For nitrogen determinations, 100 c. c. digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE XI.—*Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution*

QUANTITY (IN CUBIC CENTIMETERS) OF $N/5$ PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN

Digestion period.	Experiment No. —															
	26[a]		25[b]		27		28		30		31		32		34	
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
Hours.																
2	22.2	18.2	17.0	18.2	44.3	9.3	41.8	10.4	14.7	13.7	9.7	11.4	18.4	18.4	21.5	19.7
6	32.8	26.8	25.3	28.2	46.2	19.9	45.2	20.5	27.0	27.2	19.0	21.5	31.7	33.8	34.7	34.2
Theoretical maximum	52.4	41.9	50.5	41.8	47.1	45.3	45.6	44.5	49.3	41.3	49.1	40.1	51.2	45.6	51.3	45.1
Extractive nitrogen	8.3	8.6	10.0	8.6	6.6	8.1	8.1	8.9	7.7	9.1	8.2	10.7	7.5	8.6	7.3	8.2
Trypsin nitrogen	3.5	3.5	7.0	7.0	7.0	7.0	7.0	7.0	14.0	14.0	4.7	4.7	9.4	9.4	9.4	9.4

[a] Results obtained at end of 26 and 168 hours, instead of 2 and 6 hours.
[b] Results obtained at end of 7 and 24 hours, instead of 2 and 6 hours.

TABLE XI.—*Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution*—Continued

QUANTITY (IN CUBIC CENTIMETERS) OF *N*/5 ALKALI PROTEINATE NITROGEN

Digestion period.	Experiment No. —															
	26		25		27		28		30		31		32		34	
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
Hours.																
2	5.9	3.7	6.2	5.1	2.9	3.0	3.6	3.0	3.2	3.1	2.8	3.5	3.7	4.9	4.8	5.1
6	5.0	7.0	5.2	6.5	.9	3.6	.0	5.1	3.9	3.9	4.5	5.0	3.7	3.8	2.3	3.5
Age of meat ...days..	8	8	31	31	.....	6	.....	21	19	19	28	28	19	19	8	8
Trypsin used, gm..	1	1	2	2	2	2	2	2	4	4	2	2	4	4	4	4
Trypsin No.	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3

It is obvious that the amino nitrogen contained in the digestion fluid and actually determined was the sum of the amino nitrogen derived from (1) the trypsin; (2) the nitrogenous extractives, both of which were present before digestion began; and (3) the amino groups unlinked by the cleavage of the more complex proteoses into the simpler peptones and polypeptids. This is brought about by the action of the trypsin-sodium-carbonate solution during the digestion process. The results actually obtained in the determinations were diminished by the sum of 1 and 2, so that the figures in Table XII correspond to 3, or the amino nitrogen actually formed by the digestion. The minus quantities obtained in this way in some of the experiments for the 15-minute digestion period are probably due to the fact that the errors in determining the small amounts of amino nitrogen in 1 and 2 are large when compared with the small amount formed during 15 minutes' digestion.

DISCUSSION OF THE DIGESTION EXPERIMENTS

THEORETICAL MAXIMUM.—If the digestion of the meat by trypsin could be brought to completion, the meat proteins would be split into simple amino acids. Such a complete cleavage of protein by a trypsin sodium-carbonate solution seldom, if ever, occurs. One reason is that the action of the trypsin becomes slower and slower the nearer the digestion process approaches completion. But by boiling the meat with hydrochloric acid, as already described (p. 678), the proteins and other nitrogenous substances are completely hydrolyzed, or 100 per cent digested. The data in Table XII, under the heading "Theoretical maximum," were obtained from Table V. The total amino nitrogen obtained from hydrochloric-acid hydrolysis minus the amino nitrogen in the extractives gave the figures recorded in Table XII. A slight error was here involved; the correction should have been the amino nitrogen in the extractives after acid hydrolysis, not before. For the present purposes this error is regarded as entirely negligible.

TABLE XII.—*Rate of liberation of free-amino groups in trypsin-sodium-carbonate digestion mixtures*

Digestion period.	Quantity (in milligrams) of amino nitrogen in 10 c.c. of digestion fluid, equivalent to approximately 0.5 gm. of meat in experiment No.—															
	26		25		27		28		30		31		32		34	
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
¼ hour	.....	.....	0.08	−0.41	0.56	0.01	0.51	−0.39	−0.22	0.00	0.16	0.16	0.37	0.35	0.44	0.30
1 hour	0.09	(b)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2 hours	.20	0.25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3 hours	a .64	a .42	.72	67	1.93	.70	1.92	.73	1.43	1.42	1.39	1.65	2.76	2.94	3 08	2.89
6 hours	a .79	a .68	1.43	1.26	2.60	1.33	2.33	1.46	2.14	2.32	2.05	2.52	4.16	4.64	4.59	4.45
11 hours	.....	.....	1.94	1.82	2.67	1.73	2.71	1.93	.....	.....	.....	.....	.....	.....	.....	.....
12 hours	.....	.....	.....	.....	.....	.....	.....	.....	2.99	3.44	2.82	3.51	5.47	6 20	6.12	5.82
24 hours	1.30	1.19	a 2.74	a 2.79	3.36	2.52	a 2.93	a 3.94	3.71	3.92	3.65	5.23	7.81	8.37	8.29	7.93
48 hours	2.13	1.74	2.95	3.68	a 3.81	3.95	a 3.94	a 5.39	4.95	5.39	5.52	6.44	9.37	11.30	9.44	8.93
72 hours	a 2.35	a 2.01	2.09	3.38	4.63	4.92	a 3.82	a 5.14	.....	.....	.....	.....	.....	.....	.....	.....
96 hours	.....	.....	2.51	4.33	.....	.....	.....	.....	6.11	6.22	6.75	7.40	10.59	13.07	13.36	11.14
120 hours	2.63	2.30	3.04	5.09	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
144 hours	.....	.....	.....	.....	.....	.....	.....	.....	a 6.83	7.22	7.46	7.74	.....	.....	.....	.....
12 days	.....	.....	.....	.....	.....	.....	.....	.....	8.66	8.12	.....	.....	11.97	13.29	12.10	11.45
Theoretical maximum	11.98	10.22	11.98	10.22	(c)		(c)		12.05	10.42	11.97	10.18	12.39	10.94	12.16	.0.89
Amino nitrogen in extractives	.68	.46	.68	.46	1.11	.27	1.11	.27	.51	.53	.59	.77	.43	.41	.50	.47
Amino nitrogen in trypsin	.85	.85	1.71	1.71	1.71	1.71	1.71	1.71	3.42	3.42	.64	.64	1.28	1.28	1.28	1.28
Age of meat.....days	8	8	31	31	.....	6	.....	21	19	19	2.8	2.8	19	19	8	8
Trypsin used.....gm.	1	1	2	2	2	2	2	2	4	4	2	2	4	4	4	4
Trypsin No.	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3

[a] Results obtained one hour later than the time indicated, i. e., in experiment 26, the results were obtained for four and seven hours' digestion, etc.
[b] Determination lost.
[c] Low results obtained were rejected (see p. 680).

PERCENTAGE OF MEAT DIGESTED.—In 0.5 gm. of meat the theoretical maximum amino nitrogen varies between 10 and 12 mgm. In order to convert the figures for amino nitrogen in Table XII to the percentage of the total amino nitrogen, it is only necessary to multiply them by a factor easily obtained mentally, which factor varies from 10 to 8.5. Thus, in experiment 32, 10 c. c. of the beef sample 11 digestion fluid contained 4.16 mgm. of amino nitrogen at the end of six hours. At complete digestion 12.39 mgm. would have been present; therefore $4.16 \div 12.39$ or 34 per cent of the total amino nitrogen present had been unlinked by the cleavage of polypeptids under the conditions of the experiment. The same figure may be obtained directly by multiplying in round numbers 4 by 8. A minute before, or after, this particular amino-nitrogen determination was begun, a 100 c. c. portion of the same digestion fluid had been neutralized by the addition of 24.5 c. c. of $N/5$ sulphuric acid. This mixture was brought to a boil in the next few minutes, filtered, and total nitrogen was determined in the filtrate and the precipitate. The results were recorded in Table XI. This table shows that in the same experiment, No. 32, at the end of six hours' digestion of beef sample 11, approximately 60 per cent (i. e., $31.7 \div 51.2$) of the originally insoluble beef sample 11 nitrogenous substances had gone into solution as proteoses, peptones, and amino acids. These figures show how imperfect is the expression "Percentage of meat digested." The digestion process involves several chemical changes which take place at different rates. In general, the cleavage (by trypsin) of the larger molecules of alkali proteinate and proteose goes on at a comparatively rapid rate, the cleavage of the simpler peptone and polypeptid molecules at a slow rate. These facts are illustrated by the foregoing data of experiment 32. By the second method of measuring digestion it was shown (Table XI) that at the end of six hours' digestion 60 per cent of beef sample 11 had been transformed into proteoses, peptones, and amino acids; but by the third method of measuring digestion only one-third of the total amino nitrogen present had been unlinked (Table XII). The last two statements are correct; but it would not be entirely correct to say that according to the second method 60 per cent of beef sample 11 had digested at the end of six hours, or that 34 per cent of beef sample 11 under the same conditions had digested, using the third method of measuring digestion. A single figure can not describe several simultaneous processes in this case. The results in Tables XI and XII were obtained with the same digestion mixtures. The results in Table XII are expressed in milligrams of amino nitrogen obtained from 10 c. c. of digestion fluid, equivalent to approximately 0.5 gm. of meat.

PRESERVATIVES NOT USED.—In all the digestion experiments the flasks in which the meat was heated and later digested were partly sterilized by the heating in the boiling-water bath. During the diges-

tions in which the pepsin-hydrochloric-acid solution was used bacterial action was excluded from the digestion mixtures by the bactericidal action of the 0.2 per cent hydrochloric acid. During the digestions in which trypsin-sodium-carbonate solution was used bacterial action was not excluded, because any bacteria introduced into the digestion mixtures would not be destroyed by 0.5 per cent sodium carbonate. When the digestion period was short (Tables X and XI)—i. e., 24 hours or less—the possible error due to such recently introduced bacteria was negligible because the proteolytic action of the most vigorous proteolytic bacteria is very weak when compared with that of trypsin. When the digestion period was long enough (Table XII) the chemical changes brought about by the bacteria may have appreciably affected the results. No preservatives were used in any of the digestion experiments. This was regarded as an almost necessary condition in view of the fact that both the wholesomeness of immature veal and the influence of certain preservatives on digestion, health, etc., have been subjects of controversy. In the third method it was decided to carry on the digestions as aseptically as possible and to regard the results obtained in the first 48 hours as practically uninfluenced by bacteria. Generally after a few days putrefactive odors were noticed in the digestion mixtures. In so far as a very strong putrefactive odor can be caused by slight chemical changes in which small amounts of strongly odoriferous substances are produced, the amino determinations were made as late as 12 days after beginning the digestion in mixtures that were undoubtedly putrefying as judged by the odor. The practical necessity of a long digestion period in the third method, because of the slowness of amino-nitrogen liberation, together with the indeterminate effect of bacteria, is an objection to this method. The results of the first and second methods showed that under similar conditions mature beef and immature veal proteins were digested to the proteose and peptone stage with practically equal speed. However valuable such data may be they are not complete until the speed of the last transformation in the digestive process is measured for both. If the rate of liberation of amino groups in immature veal had been found to be slower than in mature beef, that fact would have constituted a good reason for the claim that immature veal digests with difficulty in the human digestive tract. The principal advantage of the third method as applied to digestion mixtures lies in the fact that it affords an easy, rapid method of measuring amino-nitrogen liberation, which can not easily be measured by other methods.

Graphic representation of results.—In figure 4 the results for amino nitrogen in experiment 32 are plotted. Most of the other curves obtained in this way were flatter because the rate of amino nitrogen liberation by trypsin 2 was slower. The curve for experiment 32 indicates that during the first 36 hours, approximately, the veal digested

a little more rapidly than the beef. After 48 hours the digestion mixture of veal sample 11 smelled putrid. In addition to the amino nitrogen liberated by the trypsin in this mixture non-amino nitrogen was transformed into amino nitrogen by the bacteria. This was indicated by the fact that after 48 hours' digestion amino nitrogen in veal sample 11 was higher than the amount originally present in the meat. During the bacterial and tryptic action which followed, practically all of the nitrogen was transformed to amino nitrogen. The mixture of beef sample 11 did not smell putrid in this experiment. In experiment 34, which was a repetition of experiment 32 except that beef and veal samples 12 were used, both mixtures from these samples had become putrid, and in both, as the data in Table XI show, the amino nitrogen

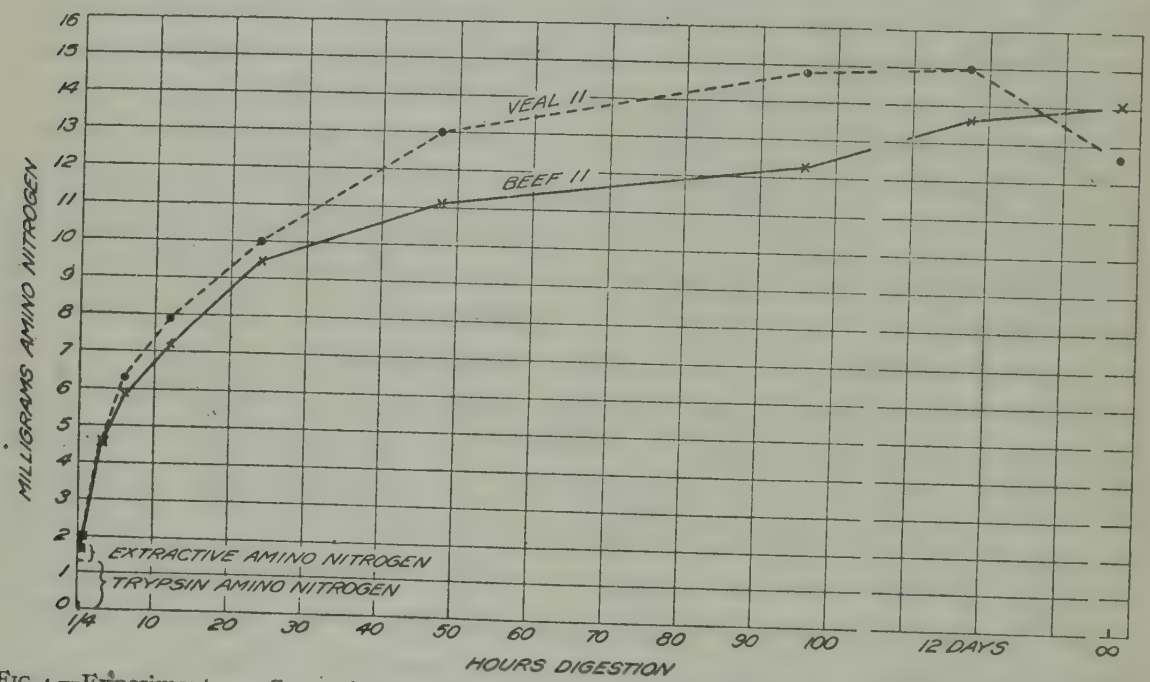

FIG. 4.—Experiment 32. Curve showing the quantity (in milligrams) of amino nitrogen in 10 c. c. of digestion fluid; used 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium carbonate plus 4,000 gm. of trypsin 3.

measured was greater in amount than that originally present in the meat. In some of the experiments putrefactive odors were not noticed, although looked for.

The general conclusion drawn from the data of Table XII was the same as that drawn from Tables X and XI—namely, that mature beef and immature veal under the conditions of the experiments were digested by trypsin with equal speed. The slight differences noticed were regarded as physiologically insignificant.

In experiment 27 and its repetition, experiment 28, veal sample 9 was compared with skim-milk sample 2, with the same object as before, to ascertain whether the method would detect a difference in amino-nitrogen liberation where such a difference existed. In both experiments, up to and including the 11-hour determinations, amino nitrogen was liberated in the skim-milk digestion mixtures much more rapidly than in veal sample 9. After this the results were somewhat irregular.

FREE AMMONIA FORMED DURING DIGESTION.—Because of the slowness with which ammonia reacts with nitrous acid (see p. 681) it was desirable to determine the amount of ammonia formed during the digestion of mature beef and immature veal and incidentally to ascertain whether the amounts formed were significantly different for the two meats. In experiments 15, 18, 20, 22, and 24, after 24 hours' digestion, 100 c. c. portions of digestion fluid, containing 0.5 gm. of sodium carbonate and corresponding to 5 gm. of meat, were transferred to Kjeldahl flasks, diluted to 500 c. c. with distilled water, and the ammonia distilled into standard acid. The mixtures were quickly brought to a boil and boiled for half an hour. This method is known to give high results, but for the purpose of comparison the errors were negligible. In all cases except veal sample 7 the ammonia obtained neutralized 2 to 3 c. c. of *N*/5 acid, amounts too small to be a disturbing factor in using the third method or indicating any differences between the beef and veal. From veal sample 7, 7 c. c. of *N*/5 ammonia was obtained. This animal was sick when purchased (see p. 675). On this score the comparatively high ammonia content of trypsin 2 was a disadvantage.

BLANKS ON REAGENTS.—It was found convenient to begin each digestion experiment with fresh alkaline permanganate solution in the absorption pipette and to make blank determinations on the nitrous-acid reagents, water, octyl alcohol, etc., before, during, and after a digestion experiment involving about 20 amino-nitrogen determinations. The blank on the reagents, allowing 20 minutes' reaction time, was 0.6 c. c. nitrogen gas when the permanganate was fresh and rose to 1.2 c. c. after this reagent had been used until absorption had become slow (see p. 680). The smallest volume of nitrogen gas measured in the beginning of a digestion experiment was 3.3 c. c.; the largest, at the end of an experiment, 28.7 c. c.

FEEDING EXPERIMENTS ON CATS

In these experiments cats of various ages were fed on a diet in which immature veal was the sole source of nitrogen.

Osborne and Mendel and their coworkers (1914, p. 334) in their investigations emphasize the difference between maintenance and growth. According to these investigators an animal can not maintain its weight unless the diet contains tryptophan, although the diet may be physiologically sufficient in all other respects. Further, an animal can not grow unless lysin is present in the diet, the amount of growth being conditioned by the amount of lysin available. Conversely, the absence of these unique amino acids results in a decline in weight or in stunted growth. According to McCollum and his coworkers (Hart, McCollum, et al., 1911), a diet properly balanced for growth may not be properly balanced for reproduction—i. e., cows fed on either the whole corn plant or the whole wheat plant would grow, but vigorous calves would be produced only by the corn-fed cows.

The principal object of the feeding experiments was to ascertain whether growth and reproduction were possible on a diet in which immature veal was the sole source of nitrogen. The data of the above investigators were used as a guide in planning the experiments.

DIET.—The cats' diet consisted of immature veal boiled for one to two hours, to which was added filtered butter fat, sodium chlorid, and calcium carbonate. The immature veal was obtained, as already described, from calves seven days old or less which were killed on the premises. When the meat was trimmed for feeding purposes, the lungs, heart, liver, kidneys, and spleen, together with adherent bits of fat, gristle, etc., were included. For the purposes of the analytic work, digestion experiments, etc., the muscle tissue alone was wanted; for the feeding the intention was to include all parts of the veal that ordinarily are eaten. Thirty-four calves were fed to the cats.

At suitable intervals of from four to seven days about 5 kgm. of veal were removed from the containers in cold storage. After being weighed the meat was cut into pieces about as large as ordinary sugar cubes, transferred to an agate-ware kettle containing about 1 liter of hot water, and boiled for one to two hours. The object was to boil the meat in a small amount of water so that it would be convenient for feeding.

Because of the low fat content of the veal, filtered butter fat was added after the boiled veal had cooled. This was obtained by melting several pounds of butter, allowing the water, casein, etc., to settle to the bottom of the containers, and pouring the supernatant fat through filter papers. The butter fat was kept in bottles in cold storage and used as required. According to Osborne and Mendel (1913, p. 424) butter fat contains no nitrogen. Funk and Macallum (1914) found traces of nitrogen in butter fat, which for the purposes of the present consideration of the diet may be disregarded.

No analyses were made of the materials fed. In a few instances the carefully trimmed muscle tissue used for analyses, etc., was included in the veal diet.

Following were the proportions of the various constituents of the diet:

Immature veal	1,300 gm.
Filtered butter fat	45 gm.
Calcium carbonate	10 gm.
Sodium chlorid	10 gm.

The last two constituents were the ordinary "chemically pure analyzed" commercial products. The diet contained no roughage. The above proportions were calculated from the data of Osborne and Mendel (1911, p. 32, 80, 86). Potassium salts and phosphates were omitted, because these were thought to be present in the veal in sufficient amounts.

After the veal had been boiled and the other materials added, the food was kept in an ice box close to the animals' cages. The gelatin present in the food caused the entire mass to become solid, so that there was no loss

through spilling when portions were transferred from the container to the smaller feed pans in the cages. Generally enough food was prepared to last from five to seven days. The ice-box compartment in which the food was kept was also used for the purpose of storing dead guinea pigs, rats, etc., for various biological purposes. Although it was desired to feed the animals with clean food, no unusual precautions were taken. The cover of the can containing the food was seldom tightly in place, and undoubtedly the food was exposed to some extent to bacterial contamination. The conditions under which the meat was kept in cold storage and then boiled were probably better than the conditions in many so-called sanitary kitchens. But the conditions under which the boiled food was stored in the ice box were certainly such as exist in no well-kept kitchen ice box. This was purposely done, in order that the diet actually fed should conform, as nearly as possible, to the poorest rather than the best ice-box conditions for food.

ANIMALS AND ENVIRONMENT.—The animals used in the experiments were ordinary cats, selected at random and brought to the animal room. Some were very young at the beginning of the feeding; others quite old. Their weights are given in Table XIII. After having lived on the immature veal diet for about six months cat 2 was crossed by cat 1, and in due time cat 2 gave birth to a litter of four kittens, given in Table XIII and in figure 6 as cats 5, 6, 7, and 8. One of the kittens (cat 7) died in a few days; the others were nursed by their mother until they could eat the immature veal. It is obvious that since both parents of these kittens had lived and grown on the immature-veal diet for 8 and 10 months, respectively, the birth of these kittens and their subsequent vigorous growth indicated that the diet was entirely satisfactory. There were no indications that toxic bodies were present in the diet or that any of the amino acids essential to normal growth were absent.

TABLE XIII.—*Description of cats used in feeding experiments*

No.	Description.	Weights.			Period of feeding.	Final disposition of animal.
		Initial.	Maximal.	Final.		
		Gm.	*Gm.*	*Gm.*	*Days.*	
1	White male kitten	695	4,080	3,220	473	Chloroformed; autopsy performed.
2	Black female kitten	837	4,040	2,620	408	Do.
3	Yellow male, old	3,605	4,940	4,070	216	Set free.
4	Black male, old	3,350	.....	3,960	50	Returned to owner.
5	White male [a]	[b] 105	.....	3,080	175	Living in a home.
6	White female [a]	[b] 110	.....	2,370	175	Do.
7	Black female [a]	[b] 95	.....	100	15	Died; marasmus.
8	Black male [a]	[b] 105	.....	2,790	175	Set free.
9	Yellow female kitten	580	.....	2,280	114	Do.

[a] Litter produced by cats 1 and 2. [b] At birth.

The animals were kept in cages, singly at first; later, after the kittens had become quite large, they were kept in pairs. The long confinement did not seem to disagree with them. All of the animals were unusually fine in their appearance and disposition, except that toward the close of the experiment cats 1 and 2 apparently suffered from the effects of the long confinement—in their case considerably over a year.

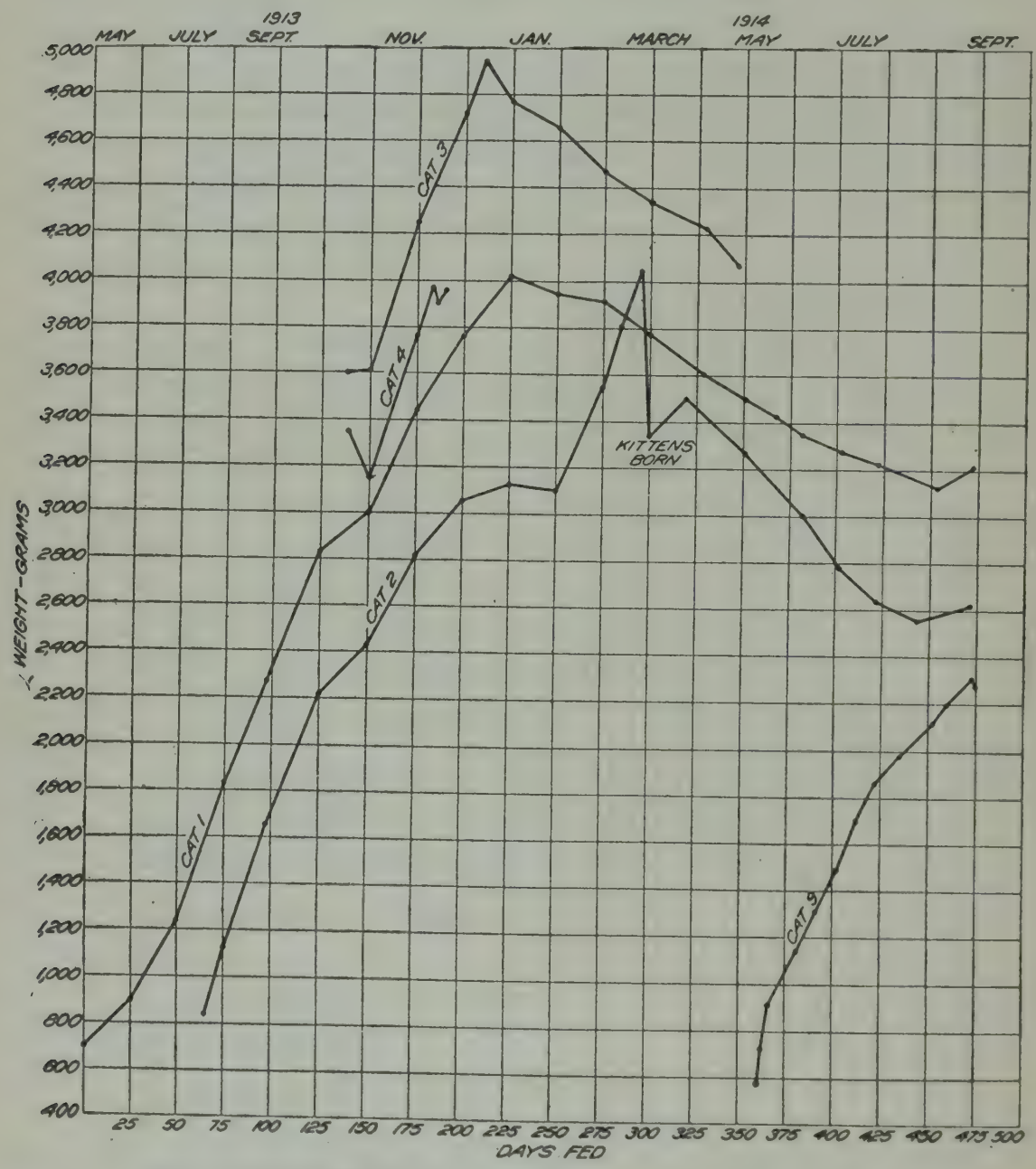

FIG. 5.—Curve showing the rate of growth of cats on an immature-veal diet.

FEEDING.—Twice every day, at 9 a. m. and 3 p. m., liberal portions of the veal food were transferred to the feeding pans and placed in the cages. The animals apparently found the food very acceptable in spite of the monotony of the diet. No attempt was made to regulate the amount of food consumed by any animal; they ate as much as they pleased. All of the boiled veal was eaten; not a single lot of the food was found to be distasteful to the animals or in any way noticeably injurious.

WEIGHTS OF THE ANIMALS.—The animals were weighed twice every week. The rapid growth of the younger animals and the fattening of the older ones are indicated in figures 5 and 6. The reason for the decline in weight of cats 1, 2, 3, and 4 in the spring and summer of 1914 can not be stated with certainty. The fact that cats 5, 6, 8, and 9, all young, gained weight rapidly on the same diet that the other cats were receiving when they were declining in weight indicated that the loss in weight was not due to the diet but rather to a seasonal variation which affected the weights of the older animals. Cats 1 and 2 were chloroformed at the end of the experiment (September 10, 1914) and autopsies performed by Dr.

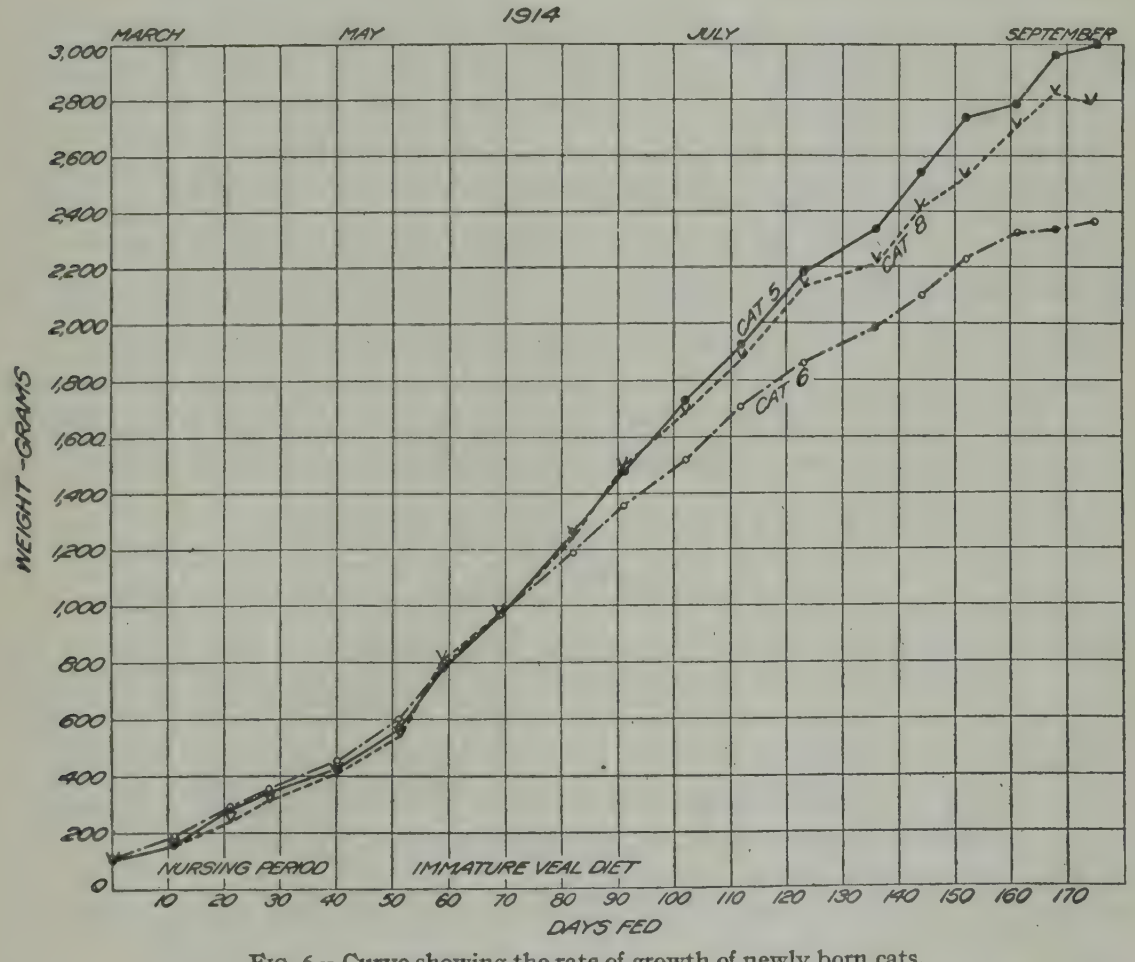

FIG. 6.—Curve showing the rate of growth of newly born cats.

H. J. Washburn, of this division. The animals were found to be in excellent condition, with liberal deposits of fat in both. Apparently the loss in weight in these two animals was due to loss of stored fat. The same was probably true of cat 3, which had the appearance of being unusually fat at the time of its maximum weight.

CRITERIA OF DIETARY SUFFICIENCY.—The excreta of the animals were not collected, nor was any chemical work done directly in connection with the feeding experiments. The ability of the animals to utilize the immature veal for the building of their tissues and for the reproduction and nursing of healthy young animals was regarded as a certain indication that the immature veal contained all the amino acids essential to

maintenance, growth, and reproduction. It is true that only one litter of kittens was born, but this would have been practically impossible had an attempt been made to maintain the parents of these kittens for two-thirds of a year on a diet lacking something essential. Cat 2 went through the period of gestation and nursing with every outward indication of excellent health.[1]

SUMMARY

(1) During the study of the chemical composition of mature beef and of immature veal, no differences between them that are physiologically significant were detected.

(2) In a large number of artificial-digestion experiments immature veal digested as fast as mature beef. The speed of digestion was measured by three different methods.

(3) Cats were fed on a diet in which immature veal was the sole source of nitrogen. The young animals grew normally on the diet; the older ones became fat. A pair of cats, after living two-thirds of a year on the diet, produced a litter of healthy young kittens which, after the nursing period, continued on the immature-veal diet with excellent growth.

(4) The work indicates that immature veal, when properly prepared, is fit for human food, especially when its deficiencies in fat and possibly in small amounts of undetermined constituents are counterbalanced in the ordinary mixed diet.

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[1] The argument has been offered that the metabolism of the fetus and of the newly born is different from that of older animals and that there is a possibility of toxic substances being present in embryonal or young tissues, which substances, though present in amounts too small to be detected by analytic methods, may be very powerful in their action upon the consumer of very young meat; or, as is sometimes alleged, the newly born animal does not excrete its metabolic end products fast enough, with the result that its tissues are loaded with waste material.

The polypeptid nitrogen which passes unused through the assimilatory system of the fetus or of the newly born is, however, not significant. If by any chance the tissues of a very young calf happened to retain some of its own metabolic products because of retarded excretion or from any other cause whatsoever, so long as the animal was normal otherwise there would be practically no danger to the consumer of such meat from poisonous end products of protein breakdown. However, the tissues of very young calves are not loaded with unexcreted nitrogen. The data obtained on this point are direct and conclusive. (See p. 673.)

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JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., JANUARY 17, 1916 NO. 16

FACTORS INVOLVED IN THE GROWTH AND THE PYCNIDIUM FORMATION OF PLENODOMUS FUSCOMACULANS.[1]

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INTRODUCTION

The experimentation reported in this paper was begun at the botanical laboratory of the University of Michigan in 1913, continued at the Michigan Agricultural College during the next year, and finally completed in 1915 at the University laboratory.

The fungus *Plenodomus fuscomaculans* was obtained from badly cankered limbs of the apple (*Malus* spp.) which were sent to the Agricultural College laboratory in March, 1911, from Boyne City, Mich. Examination of the cankers at the time of receipt and field studies during the same month showed that the trouble was different from any of the described apple diseases. The cankers showed constant association with a pycnidium-forming fungus. This organism was obtained in pure culture from a single spore, and the causal relation of the fungus to the canker was proved by repeated inoculations and reisolations. A study of the organism, both on the host and in pure culture, showed that it was a Phoma-like member of the large group Sphaeropsidales, and it corresponded to the species described by Saccardo as *Aposphaeria fuscomaculans*.

The pycnidia, however, show morphological characters by which it is possible to segregate this fungus from the larger, poorly defined genus. These characters, which may be found in the material from the host, become very pronounced in culture. The pycnidia are more or less irregular in shape. The fruiting layer is usually folded so that the chamber is recessed instead of being smooth and regular. The pycnidia are beaked. The wall is composed of two distinct layers and is complete, even at the basal portion. It seems proper to emphasize the morphological character of the wall. Accordingly, the removal of this species from the genus Aposphaeria Berk., and the placing of it in the genus Plenodomus Preuss,

[1] Published with the permission of the Director of the Michigan Agricultural Experiment Station.

is proposed. The name of the fungus becomes, under this arrangement, *Plenodomus fuscomaculans* (Sacc.), n. comb.[1]

The present paper deals wholly with the physiological phase of my investigations, the phytopathological studies being reserved for another paper.[2]

The problem consisted of the investigation of the relations of the organism to the environment and the fitting of the environment to the organism—a marked reversal of the common practices in culture making.

HISTORICAL REVIEW

The history of the cultivation of micro-organisms is linked with the history of bacteriology and mycology. Progress in these sciences has been largely due to the clarifying effect of pure-culture methods. These originated with the discovery of the method by which media could be sterilized. It is a significant fact, and one which can be traced to the influence of these early experiments, that the solutions and materials used in the first crude cultures were the highly concentrated vegetable and animal decoctions and infusions which experience had shown to be highly liable to putrefaction. Mycology made great advance when, utilizing the newly discovered methods of isolation, the various groups of organisms were brought into pure culture by such masters as Brefeld, De Bary, Hansen, and Zopf. The earlier methods are in vogue to-day in the great bulk of mycological or applied work. In the cultural work of these pioneer studies nutrition was the only factor to which consistent attention was given.

The influence of other factors than nutrition was recognized early, but the methods of culture were varied but little to fit these conditions. Pasteur (1861)[3] showed the difference between aerobiosis and anaerobiosis, but this distinction long remained obscured by the problems of fermentation. The oxygen relations of fungi have been neglected in the ordinary cultural technique, since most fungi tolerate the conditions of the plugged flask or test tube. The sharp temperature requirements of some animal pathogens focused attention upon this factor very early, and accordingly incubators and devices to furnish constant temperature were developed. But there has been wide neglect of this factor. That bacteria grow best in a medium slightly alkaline to litmus and fungi in a medium slightly acid and that this difference can be used to advantage in isolation early became dicta of the sciences. The growth of organisms takes place within such wide limits in composition of culture media and

[1] A discussion of the morphology of this fungus was prepared for the 1915 Report of the Michigan Academy of Science. Delay in publishing this report makes it necessary to give the proposed change in nomenclature in this connection, with only a summary of the reasons for making the change. The latter publication may be looked to for a more complete account of the morphology of the fungus.

[2] The physiological work was suggested by Dr. C. H. Kauffman, of the University of Michigan, and has been done under his direction. I am also indebted to Dr. E. A. Bessey, of the Michigan Agricultural College, for advice and help throughout the investigation.

[3] Bibliographic citations in parentheses refer to "Literature cited," p. 766–769.

under such a range of conditions that accordingly these environmental factors have been neglected in culture work.

The emphasis placed upon nutrition has developed a great body of facts regarding media in which organisms will grow and rules for the preparation of the media. These compositions have the common characteristic that for the most part they present highly concentrated food supplies so complex as to defy analysis. The list includes beef infusion, prune juice, wort, Nähr solution, bread (plain or soaked in sugar solutions), vegetables of all kinds, and the long list of nutrient hydrogels. These media have given excellent vegetative growth; but if the common molds are excluded, it may be said that on the majority of media fructification is the exception rather than the rule.

In recent years many kinds of fruits, vegetables, and other biological products have been tried, either directly or as a base for a nutrient hydrogel. Some of these have produced fructification in forms which had previously grown only vegetatively in culture. Notable examples are corn meal, or corn-meal agar, which in the hands of Shear (Shear and Wood, 1913) and others led to an unraveling of the Gloeosporium complex, and oat agar, which in the hands of Clinton (1911) solved the historic *Phytophthora infestans* difficulty.

The complexity of the vast majority of combinations used in contemporary research, however, does not permit the analysis of the contributing factors which lead to fructification. The net contribution, therefore, toward a final analysis, which would furnish a key for unlocking closed approaches with other organisms is small, and further advance, so far as indicated by such work, must be by the same wasteful method of haphazard trial. It is known that organisms will grow under a vast assortment of conditions, but very little is known of the conditions which call out any particular phase of development.

Our knowledge of the physiology of micro-organisms has largely come from a study of their behavior under controlled conditions. The very analytical nature of the type of research used in the study of metabolism has made its methods in sharp contrast with those just described and has made possible evaluation of the various factors involved. The pure-culture methods just discussed and researches on the metabolism of micro-organisms have progressed side by side, and only slightly have the basic principles of the latter been influential in determining the course of the former. The art of cultivating organisms has indeed been developed, but this work is almost wholly empiric; although there is a mass of fundamental facts dealing with metabolism and with the reactions of plants to their environment, these for the most part are totally ignored in ordinary culture methods (Benecke, 1904; Behrens, 1904, p. 436–466).

Studies of the effects of various factors upon the metabolism of fungi naturally were made first with the nutrition of the micro-organisms. It was essential that the work be done with synthetic media; and along

with the development of the various synthetic culture solutions our knowledge of the nutritional requirements of micro-organisms has arisen (Pasteur, 1858, Raulin, 1869, Nägeli, 1880).

The gradual extension of the point of view of physiological response may be considered a guiding principle in cultivating organisms, and after a period of more or less accidental or random application of specific environments to influence growth or reproduction, a definite method based upon this teaching has been developed. Roux and Linossier (1890), with the animal pathogen, *Dematium albicans*, secured marked reactions to specific environmental factors, especially nutrition and oxygen. At the same time Winogradsky (1891) began his well-known work with the nitrifying organisms which he isolated by his method of "elective culture." This method, which consists essentially of so establishing the environment that only organisms of the desired type are able to develop, was carried to great perfection by Beijerinck (1901) with his similar "intensification" method. The bacteria and algæ with which Beijerinck worked required or tolerated different amounts of free oxygen, different nutrition, especially mineral salts, and different temperatures. Beijerinck used these differences as a means of isolation of various forms from a complex substratum (Stockhausen, 1907).

About the same time Klebs began his work on algæ and fungi in pure culture. Where others were concerned with growth, Klebs (1896) made the pure culture answer unsolved questions of life history. He (1913) recognized in the organism definite potentialities—the heredity of the organism. The manifestations of these potentialities are seen in the reactions to environment and in the limits of the various factors tolerated. The particular line of development followed by the organism can be traced to conditions outside of the potentiality, either inner conditions inaugurated by the environmental complex or outer conditions which work through their ability to set up certain internal effects. From this line of reasoning it was but a step to the position that the development of an organism is the resultant of the environment working upon definite internal potentialities of the organism and that with a given potentiality the same external conditions call forth the same response with the constancy of a chemical reaction. This response may be predicted from the type of conditions given, and in this regard Klebs (1900) announced the following propositions, as based upon his work:[1]

1. Growth and reproduction are life processes, which among all organisms depend upon different conditions; among the lower organisms, probably external conditions determine whether growth or reproduction ensue.

2. As long as the characteristic outer conditions for the growth of the lower organisms are present, reproduction does not set in. The favoring conditions for this process are always more or less unfavorable to growth.

[1] Author's translation.

3. Growth and reproduction differ also in that the working limits of the general life conditions, temperature, oxygen, etc., are narrower for reproduction than for growth. On this account growth can still take place, even if reproduction be limited through too weak or too strong influence of some factor.

4. Growth appears mostly as a preliminary for the initiation of reproduction, and, therefore, as an inner condition for it. Up to a certain limit, not directly growth, but the longer assimilation period is determinative.

From this point of view all the factors which influence life may be considered, and from the basis of the knowledge of their effects on growth, the ultimate effects of these factors upon reproduction may be predicted more or less accurately. This Klebs (1900, 1904) has done in the summary of his contributions to the physiology of reproduction.

Since that time research along this line may be divided into two types of endeavor: (1) Extending the groups to which the laws may be shown to apply and (2) the critical testing of the conclusions with the very organisms with which Klebs worked. The former has extended the limits so that none of the great groups of fungi or algæ are without many examples of the application of the conclusions. The work of the second type has opened up new points of view. Klebs in his experiments used a single strain, and the common experience, in repeating his experiments, is failure until the limits and life relations of the particular strain at hand are known. Accordingly, Kauffman (1908) has emphasized this point in his work with the same species of Saprolegnia that Klebs used; but where Klebs worked with one strain, Kauffman used two additional ones; and with this number of forms, each an entity and each varying from the other, Kauffman was able to show that within the limits of each the conclusions were valid. This work emphasizes a point which Klebs has made for his various forms, that each is a specific potentiality, but it makes the specific potentialities innumerable in their scope.

The particular organism with which I worked was one closely related to the large genus Phoma. This group, although containing many species, some of great economic importance, had received little attention from a physiological point of view. There have been no attempts to test the validity of Klebs's conclusions for the Sphaeropsidales.

Ternetz (1907) isolated from the roots of species of Vaccinium and Oxycoccus a series of *Phoma* spp. suspected of being mycorrhiza-producing forms. These organisms were grown in pure culture on synthetic media, and their relations to oxygen, nitrogen, and mineral salts were determined with great care. They were found to be sensitive to a restriction of the oxygen supply, especially when growing in a medium poor in nitrogen. These organisms were shown to have the power of utilizing nitrogen from the air. Saida (1902) has claimed the same for *Phoma betae*.

Later, König, Kuhlman, and Thienemann (1911) cultured a species of Phoma isolated from water, and although they secured pycnidia in a few

instances, they were unable to determine the conditions under which fruiting bodies developed, but they surmised that probably the lack of food supply was the causal relation.

Other related genera have been studied more or less, and detailed accounts of the growth and fruit-body formation of several species of Phomopsis on the ordinary laboratory media have been given. (Roberts, 1913; Harter and Field, 1913; Harter, 1914.)

Plenodomus destruens has recently been described by Harter (1913), who has cultured the organism upon the ordinary laboratory media, and has determined its optimum temperature. For the most part the above-mentioned articles, written from a phytopathological point of view, have used the pure culture as a device for furnishing material for pathogenic studies, and the description of the organism in culture is largely for diagnostic purposes.

METHODS OF INVESTIGATION

As *Plenodomus fuscomaculans* had shown no form of reproduction under the ordinary methods of culture (see p. 724), it seemed to afford an excellent opportunity to try the effect of various environmental factors as a test of the applicability of the methods of Klebs to phytopathological studies.

The strain of the organism used was the progeny of a single pycnidiospore, isolated by the dilution method. This strain had been tested and was known to be pathogenic to apple. In 1913 another isolation was made from a second collection of material, and a second strain obtained and similarly tested. In all later work both strains were used in all experiments. Aside from slight differences in vigor of growth, the cultures gave the same reactions.

All experiments were made in duplicate with each strain; hence, the experiments reported give results which are a summary from the record of at least two, and, in most cases, of four parallel cultures.

The glassware used, unless otherwise indicated, was the ordinary German glass. All glass culture dishes, when other than tap water was to be used, were cleaned by immersion overnight in cleaning fluid, followed by four rinsings of tap water and one rinsing of distilled water. When water of a higher purity than ordinary distilled water was to be used in the medium, the vessels were given an additional rinsing with the purer water.

The most commonly used culture dishes were small glass preparation dishes, or capsules, of about 35 c. c. capacity. These had a loosely fitting cover which rested upon a shoulder of the bottom.

The chemicals used were those of Kahlbaum. Solutions of various chemicals were made up as weight-normal solutions (1 molecular weight in grams in 1 liter of water); and where chemicals contained water of crystallization, this was added in computing the molecular weight.

The various nutrient media mentioned were made according to the ordinary formulæ. Prune-juice agar was made by using 75 gm. of prunes with 20 gm. of agar per liter. Pea, corn, and oat broth were made by autoclaving two seeds or grains of each in 10 c. c. of distilled water.

The tap water used in some experiments had a conductivity of approximately 400 to 600×10^{-6}, while the conductivity water averaged 2×10^{-6} at the time of preparation. This water was obtained either by distilling ordinary distilled water in a block-tin still or by double distilling such water in Jena glass. As is generally recognized, ordinary distilled water varies greatly in quality, but the conductivity of the distilled water used was probably within 4 to 12×10^{-6}.

The filter paper used was Schleicher and Schull's, and, unless otherwise given, was No. 595. All media were autoclaved at approximately 15 pounds for 10 to 15 minutes, unless otherwise stated.

Inoculations, unless specified otherwise, were made with one drop of a spore suspension obtained by crushing pycnidia in a water blank. This was then filtered through a filter paper into a sterile test tube. The filter paper was sterilized in a test tube drawn out to make a funnel. This gave a device by which large masses of mycelium and pycnidia walls could be strained from the suspension. The spore suspension was added to the various cultures by means of a sterile bulb pipette equipped with a long, small-bore outlet.

EARLY EXPERIMENTS WITH ORDINARY LABORATORY METHODS

The organism brought into pure culture was grown upon ordinary laboratory media. This work was done in the spring and fall of 1911 at the Michigan Agricultural College, at a table at the rear of a large laboratory lighted from one side. Cultures were made in Petri dishes, flasks, and test tubes. Standard agar, prune-juice agar, apple stem and bark agar, apple twigs, parsnips, corn meal, potato, carrot, bean pods, beef broth, and filter paper, without other nutrients, as well as with various nutrient solutions, were the media employed. Cultures were grown under a variety of conditions, such as room conditions (test tubes in cans or in wire baskets), in the incubator at 25° C., and in the ice box at temperatures ranging from 7° to 13°. A few cultures were grown at 37.5°. On all the media mentioned growth was obtained, with more or less difference in color or vigor, but in no case were fruiting bodies of any sort produced. In some cases the cultures were allowed to dry out gradually; in other cases sterile water was added from time to time. Flasks of corn meal, with an abundant water supply, were set away in a cupboard for three months in an attempt to secure fruiting bodies in the time-honored way. In spite of this variety of trials, the organism remained a typical "sterile fungus," of which a number have been reported in literature.

But the organism, when inoculated into the host, gave characteristic lesions and typical pycnidia from which the organism could again be isolated. These reisolations were repeatedly tested, with results parallel to those obtained from the parent culture. Certain fungi—e. g., *Botryosphaeria ribis* and *Rhizoctonia* spp.—are known to fruit exclusively upon the host, and evidence seemed to point to this organism as one of that type.

EXPERIMENTS UNDER CONTROLLED CONDITIONS

In 1913, experiments were begun at the University of Michigan laboratory. In this work an attempt was made to find the effects of varying environmental factors, or, in other words, to analyze the formative as well as the inhibiting factors involved in growth and reproduction.

CONDITIONS FOR GROWTH AND REPRODUCTION

Physical Factors

LIGHT

The influence of light upon organisms has been recognized for a long time. Fries (1821) and the early authors attributed great morphogenic power to light. They found their greatest substantiation of the effect of light upon organisms in the excessive growth of mycelium in caves, accompanied, as it was, by the suppression of fructification. The literature is full of these observations, many of which are quoted by Elfving (1890). Scientific experiment with light as a factor influencing growth and reproduction of fungi began with the classic studies of Brefeld (1877, 1881, 1889) on *Coprinus* spp. Brefeld found in some species a complete suppression of fructification when cultures developed in the dark; in other species fructification took place, but the growth was puny. In some the high temperature of the summer replaced in part the beneficial effect of light. In a set of interesting experiments Brefeld showed that the exposure of mycelium to light need not be long (two to three hours) in order to have fructification begin, and that cultures so exposed developed normally, although in the dark. The work of Brefeld substantiated that of the older observers. Lakon (1907) has attempted to show that the action attributed to light is really due to transpiration differences in the cultures of *Coprinus* spp.

Downes and Blunt (1878) had previously experimented with the effect of light upon bacteria and found that it had a very detrimental effect upon these organisms. This they attributed to the action of the ultraviolet rays in augmenting oxidation, a property of light long recognized by chemists. Their conclusion was later substantiated by Ward (1893).

Elfving (1890) gave the results of his experiments with light in a monograph on the subject. Searching the literature, the only important experimental work found was that of Brefeld (1877, 1881, 1889) already

mentioned. Many had studied the effect of light upon germination, but the varying intensities of light used, etc., yielded nothing in the way of a generalization.

Elfving (1890) sought to find the influence of light upon metabolism. He used cultures of *Penicillium* spp. and a related fungus (*Briaraea* sp.) growing in a synthetic solution. He used several sources of carbon and nitrogen. Basing his conclusion upon the dry weights obtained in the light and in the dark, he decided that light acts upon fungi as an inhibitor of organic synthesis. The closer the food material is to protoplasm in its make-up, the less the light inhibits. This produces the result which he finds analogous to conditions in the higher plants—that light restricts vegetative growth. Elfving, in view of the great similarity of fungi in their physiological relations, boldly makes his conclusions apply to the whole group of fungi.

Lendner (1896) tested the effect of light upon species of Mucor, Botrytis, Amblyosporium, and Sterigmatocystis, finding that light was effective only under conditions of unfavorable nutrition.

Finally, in the experiments of Ternetz (1900) with *Ascophanus carneus*, asci were produced only under the influence of light.

Light is seen to be a factor of widely varying importance for organisms, although the effect on vegetative growth is commonly shown to be prejudicial. For some it is a morphogenic factor of great influence; for others it is of no moment.

Pure cultures of the organism on prune-juice agar and on parsnip had been brought from the Agricultural College laboratory. At Ann Arbor these cultures began to produce pycnidia in a few days. When analyzed, this striking behavior showed that light was probably the factor concerned with the fruit-body formation. The following experiments were started to test the validity of this inference. While work at the Agricultural College had been done some distance from the window (25 to 30 feet), the cultures at Ann Arbor were placed a few feet from a south window in strong diffuse daylight, and at times in direct sunlight.

Experience had shown that the organism would make a fair growth on filter paper. Filter-paper disks, about 5 cm. across, were folded to form cones, and these were set up in 10 c. c. of tap water in preparation dishes. These were autoclaved. To some, one drop (1/20 c. c.) of a sterile *M*/1 chemical was added, as indicated in Table III. The preparation dishes were inoculated with a mycelium suspension, and were placed in tall battery jars covered with filter paper. One set of cultures was placed in a light-tight cupboard, while the other was left upon the table in strong diffuse light. Thermometer readings showed at times of strongest light that the illuminated cultures were 2 degrees centigrade warmer than those in the dark. Readings were made in nine days.

TABLE I.—*Effect of light: Tests with filter paper (readings in 9 days)* [1]

Conditions.	Number of pycnidia.	Growth.
Filter paper in light	+	++
Filter paper in dark	−	++ [2]

[1] In tables where a single plus symbol (+) is contrasted with the negative sign (−), presence or absence is meant. Where a series of readings is given and several plus symbols are used with reference to pycnidia production, they give the average of two and at times of four readings, as follows: + = 1 to 10 pycnidia; ++ = 10 to 25; +++ = 25 to 50; ++++ = 100. As applied to growth the same plus symbols mean, respectively, scant, fair, good, abundant growth.

[2] A trifle stronger than above.

The cultures which had been in the dark were exposed to light about an hour at a time, when the reading was made. A second observation after 27 days showed the following result:

TABLE II.—*Effect of light: Tests with filter paper (readings in 27 days)*

Conditions.	Number of pycnidia.	Growth.
Filter paper in light	+	++
Filter paper in dark (except one hour's exposure)	Sclerotia.	+++

These bodies, called provisionally "sclerotia," when examined under the microscope were found to be minute brown bodies about one-tenth the size of the ordinary pycnidium and consisted of a firm, solid pseudo-parenchyma.

In no case was any suggestion of chamber formation noticed; nor were any spores found. It is noteworthy that the growth after this longer period could be seen to be stronger in the dark than in the light.

As part of the same experiment, a drop of some sterile *M*/1 chemical was added as indicated to a number of similar filter-paper cones. The results are as follows:

TABLE III.—*Effect of light: Pycnidium formation on filter paper plus various chemicals*

Chemical.	9 days.		27 days.		40 days.	
	Light.	Dark.	Light.	Dark.	Light.	Dark.
Filter paper + approximately 1/20 c. c. of—						
Calcium nitrate, $Ca(NO_3)_2$ *M*/1	+	−	+	Sclerotia.	+	Sclerotia.
Potassium acid phosphate, KH_2PO_4 *M*/1	+	−	+	−	+	+
Potassium nitrate, KNO_3 *M*/1	+	−	+	−	+	Sclerotia.
Calcium acid phosphate, $Ca(H_2PO_4)_2$ *M*/1	+	−	+	−	+	−

At the time of making the first reading, the cultures were exposed to the light for about an hour, and at the second reading they were exposed to strong diffused daylight for two hours.

From a consideration of the experiments reported in these tables, it is evident that light is a factor directly concerned with pycnidium production. There is also a strong tendency toward increased growth in the dark.

The experiment has been repeated many times, with a great number of duplicate cultures (60 in one instance), and always with similar results. The following is a typical experiment. Preparation dishes with water distilled out of sulphuric acid and filter paper and with water alone were inoculated with spores of each of the two strains of the organism. One set was wrapped in a double thickness of paper such as is used in photographic film rolls. The dishes exposed to light were set in glass battery jars on the window sill. The light was made diffuse by a sheet of yellow manila paper tacked on the window. The dark cultures were set away from the window in the interior of the room. The difference in temperature was the reverse of the conditions in the preceding experiments, since closeness to the cold window more than compensated for the effect of the light. In this experiment after a month no pycnidia formed in the dark, while in every culture in the light numerous pycnidia were found.

TABLE IV.—*Effect of light: Test with two strains of the organism*

Strain and conditions.	Pycnidia.		Growth.	
	Light.	Dark.	Light.	Dark.
Strain I:				
Filter paper + water	25	0	++	++++
Double-distilled water	2	0	+	+
Strain II:				
Filter paper + water	11	0	+	+
Double-distilled water	0	0	+	+

To avoid the criticism that the results observed were due to differences in aeration brought about by wrapping the capsules, or by the use of the dark closet, and to test other conditions of food supply, cultures were made with corn broth, and these were placed in a specially constructed light-tight box, which, however, allowed aeration. The box was made of two tubes of different diameters (7 and 9 inches), one inside the other. These cylinders were each 12 inches tall and toothed at the ends. A pair of caps were made for these cylinders. The caps consisted of a disk of paper about 10 inches in diameter, and a short cylinder 8 inches in diameter was glued to it. The joint was made light-tight with black paraffin. When these tall cylinders were set up with the cylinders of the caps fitting between them, light was excluded. The cultures were

placed in battery jars. The toothed tops of the cylinders allowed a circulation of air. For tests with light, the cultures were ordinarily placed in a battery jar and covered with filter paper or cloth to protect them from dust. As a further safeguard from error, however, a similar container was made, but with celluloid substituted for black paper.

The result with corn broth, after three weeks, is given in Table V.

TABLE V.—*Effect of light: Test with corn broth in light-tight box*

Conditions.	Pycnidia.	Growth.
Light in battery jar	+++	Fair.
Light in celluloid chamber	++	Fair.
Dark in black-paper chamber	−	Strong.

From this experimentation it is evident that light is a determining factor for pycnidium formation in this organism, irrespective of the type of nourishment, and that the action of light is distinct from effects which might be attributed to faulty aeration in the darkened cultures. The slight depression of pycnidia formation in the slightly darkened celluloid chamber is significant. Growth is increased in the dark.

Cultures on corn broth, in both light and dark, were subjected to a variety of air conditions. Stoppered flasks were fitted with two glass tubes, one of which extended to the surface of the culture, the other merely through the cork. As indicated in Table VI, some were connected with the water pump and filtered air which had bubbled through water was gently drawn through. As a check, some flasks were left with no additional circulation, while some were plugged with cotton.

TABLE VI.—*Effect of air circulation: Test with corn broth in stoppered flasks*

[Time, 1 month[1]]

Conditions.	Pycnidia.	Growth.
Attached to aspirator:		
Light	++++	++
Dark	−	++++
Air only through small tubes:		
Light	++	++
Dark	−	+++
Flasks plugged with cotton:		
Light	++++	++
Dark	−	+++

[1] The experiment was continued a second month with no change in relative values.

This experiment eliminates any possibility that the effect attributed to light may have come from faulty aeration or deficient transpiration. The experiment further has significance from the point of view of aeration.

The production of sclerotia, as recorded in Tables II and III, after a short exposure to light, and the production of pycnidia in one case, where the exposure was not more than two hours, suggested that the exposure to light did not need to be of long duration in order to produce its morphogenic effects. The capsules of a preceding experiment, which had shown no pycnidia after three weeks in the dark chamber, were divided into series, one of which was exposed to strong diffuse light on the window sill for two hours, while the other series was continued in the dark box. The exposed cultures were returned to the box, and after a week the cultures were examined.

TABLE VII.—*Effect of light: Continued test with corn broth*

Corn broth.	Mature pycnidia.	Growth.
Dark	0	Aerial growth.
Dark, light (2 hours), dark	3–4	Aerial growth checked, mycelium matted.

Pycnidium production had not increased upon a second examination a week later.

This experiment teaches that pycnidium formation is not only associated with light, but that the effect of light is to inaugurate a type of growth which can proceed to completion even in the absence of light. But after exposure to light the number of fruiting bodies formed is limited and the process does not continue to the production of a large number of fruiting bodies.

To summarize the results of this series of experiments, it may be pointed out that light is a decisive factor, which determines, in certain cultures, whether reproduction takes place or not, and that the action of this factor is irrespective of the richness or the poverty of the substratum in nutrients. As a morphogenic factor, its action is to inaugurate fruit-body formation, but it is not essential to the process, once inaugurated. Associated with its effect in initiating reproduction, we have its repressing effect on growth.

All subsequent cultures made with the organism had good exposure to strong diffuse light, unless otherwise expressly stated.

TEMPERATURE

It has been said that the influence of temperature was very early recognized in its influence on the life processes of fungi. Raulin (1869) in his studies of *Aspergillus niger* grew the organism at the most favorable temperature—33°. Wiesner (1873) very early formulated the behavior of *Penicillium glaucum* by a law which took into account that the time necessary for fructification did not depend wholly upon the

temperature at which a culture was placed, but depended also upon the temperature at which the organism had developed, which is, of course, a way of saying that the process of fruit-body formation is a process which depends upon the previous metabolism, and that conditions which delay the latter react similarly upon the former. The literature teems with individual facts about the temperature relation (Behrens, 1905, p. 444–449). The temperature relation, better than any other, shows the significance of the cardinal points in relation to life processes. Accordingly, we have the generalization of Klebs (1900), that the limits permitting vegetative growth are wider than those permitting fructification, and this law is nowhere more admirably illustrated than in the temperature relation.

My early experiments with temperature are not applicable, because light was excluded. Experience had shown that pycnidia were formed at the ordinary limits of room temperature. Successful cultures on various sorts of media were made in the winter with the average room temperature, 20 to 23°, and in the summer with a temperature range from 25 to 30°, so long as the light factor was not neglected.

A series of temperature experiments was made with the synthetic solution described upon page 752 in 100 c. c. flasks. These flasks were inoculated, and after three weeks' growth in weak diffuse light were subjected to the temperature indicated.

TABLE VIII.—*Effect of temperature*

Temperature.	How obtained.	Number of pycnidia.	Increase in growth.
°C.			
6–6½	Constant temperature ice box with glass doors	o	Slight.
10–12	Located at window in cold hallway	+	Fair.[1]
20–22	Room temperature near window	+	Strong.
23	Constant temperature incubator, outer door open, glass door closed	o	Weak.
33	....do	o	Do.

[1] Pycnidia began to form after a week.

The varying conditions in this experiment make necessary some interpretation for the clearing away of the apparent contradictions in the results. The absence of pycnidia in the 23° and 33° incubators, which is in seeming contradiction to the production of pycnidia in the summer time, or even at ordinary room temperature, was doubtless due to the fact that either the light was too much reduced or the air was depleted of oxygen. That the former influence was not operative seems likely from the fact that cultures standing in battery jars upon the incubator had at another time produced pycnidia. The incubators contained other cultures at the time of the experiment, and, although the doors were opened from time to time, the chamber had the ordinary strong odor of old cultures. The constant low-temperature chamber

which was designed especially for this work seems free from this criticism, since cultures placed in it before icing began developed pycnidia. This incubator had two openings (1-inch diameter) to the outside and a small fan, driven by a motor, which continuously brought about good aeration and prevented fogging of the doors. The constancy of temperature during the first week can be vouched for within the limits set, and for the next month no large deviation occurred.

The lack of apparatus to give constant temperatures, and at the same time illumination and aeration, prevented any further experimentation along this line. Pycnidia have been obtained in cultures with a temperature range of from 10° to 30° C. No pycnidia were obtained at 6° C. and no other inhibiting factor than temperature is known to have entered. The experiments with the constant-temperature incubators are disregarded because of the entrance of other factors, but are included merely to show the difficulty of experimenting with this factor.

The wide limits of pycnidium production, so far as temperature is concerned, allowed great leeway in experimentation; but outside these limits temperature may show as marked an effect as light. It is noteworthy that growth shows wider temperature limits than reproduction.

AERATION

The oxygen relation is no doubt the most essential of all life relations, and the statement "No life without air" has been shown to be universal, the contributions of Beijerinck (1893), as well as those of Fermi and Bassu (1904, 1905), showing that even the strictest of known anaerobes require minute traces of free oxygen. The relation of oxygen to plants was recognized almost from the beginning, but the interpretation of respiration by Pfeffer (1889) is fundamental. In this we have respiration portrayed as the energy-releasing process. Subsequent work has dealt with the effect of various external conditions upon the respiratory quotient. Necessarily all respiration relations depend upon the quality of the nutrition as well as the quantity of nutrients. The general conclusion which has been expressed by Beijerinck (1899), that all plants have a definite oxygen optimum and that aerobes are those whose optimum is high, while anaerobes are organisms whose air requirement is low, seems to summarize most nearly the numerous contributions.

The limiting effect of scanty aeration upon reproduction has already been mentioned. Determination of the potency of this factor in any but general ways is difficult, because of other factors involved.

Observation very early showed that greater pycnidium production took place in a capsule or Petri dish than in a plugged test tube, and that small test tubes were not so effective for pycnidium production as larger ones. Similarly, when capsules were piled one on top of another in a battery jar, pycnidia production took place in the top capsules first, although in a few days or a week pycnidia were formed in all.

If a vigorous culture on suitable media (prune-juice agar or corn-meal agar) was sealed with sealing wax no pycnidia were produced, even though comparison tubes unsealed produced pycnidia in abundance. Sealed tubes which had remained without pycnidia for two weeks had the sealing wax removed, and the pycnidium formation was slowly inaugurated. Corn broth in capsules, if covered with a small bell or if placed in a battery jar with a tight-fitting ground-glass cover, produced scanty mycelial growth but no pycnidia.

Tests for aerotropism were made with spores in melted agar. Melted agar was heavily sown with spores of the organism. Some tubes were prepared with a lighter seeding. Small drops of these agars were placed on sterile slides and sterile cover glasses pressed down upon them. Other preparations were made with the cover glass tilted, as in Beijerinck's (1893) well-known experiments. These slides were put away in a moist chamber for 24 hours at ordinary room temperatures. The results of these tests were extremely interesting.

Where the spores were numerous those at the center of the preparation showed no evidences of germination other than a slight swelling. Outside the center zone germination became more and more evident. About 5 mm. from the edge of the cover glass the germ tubes were found to be 10 to 50 times the length of the spore. At the edge of the cover glass the germ tubes had extended outward nearly a half of a millimeter. Where the spores were fewer in number the germination in the center sometimes proceeded to the extension of a short germ tube. There was no evidence of a definite tropism toward the border of the cover glass, but frequently the same spore would have sent out two germ tubes from opposite sides, one growing toward the edge of the glass, the other growing inward. Then it was noticed that the sprout growing in the medium with the richer oxygen supply was from 4 to 10 times the length of the other germ tube.

Where a clump of spores occurred about halfway from the center to the edge of the cover glass, those spores near the edge swelled strongly and put out germ tubes, while spores of the same clump, situated nearer the center, remained dormant, or at least swelled only slightly. The repression of germination in these spores seemed to be related to the scanty oxygen supply, and for this there was strong competition.

A series of flasks of different sizes was prepared with filter-paper cones, wet with 5 c. c. of distilled water. These were autoclaved and inoculated with a spore suspension. Immediately after inoculation the cotton plug was pushed slightly down the neck of the flask and the flasks were sealed with melted paraffin. The flasks were set in a window in even, diffuse illumination. After a month the reading shown in Table IX was obtained.

TABLE IX.—*Effect of aeration: Test with flasks of different sizes*

Size of flask.	Number of pycnidia.	Growth.
C. c.		
50	.....	None.
100	.....	None.
250	.....	Doubtful.
500	0	Weak.
1,000	0	Fair, mycelium blackish.

There were no checks in this experiment, but the behavior of this organism on filter paper had been so constant as to leave little doubt of repression of pycnidia having taken place, owing to the sealing of the flasks.

A similar experiment was performed with a number of nutrient solutions, some of which were known to allow pycnidium production, and others of which were known to yield only strong growth. Ten c. c. of each solution were used. This experiment was done in duplicate and was carefully checked. Inoculation was made with small masses of mycelium. The flasks, after inoculation, were sealed and stood in strong diffuse light upon a table. Table X gives the summary of this experiment.

TABLE X.—*Effect of aeration: Tests with various nutrient solutions*

[Time, 1 month]

Solution.	Size of flask.	Sealed.		Check.	
		Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
	C. c.				
Raulin solution [1] (levulose substituted for sucrose).	900	0	Heavy mat.....	0	Heavy mat.
	500	0	Heavy mat.....	0	Heavy mat.
	125	0	Fair.....	0	Heavy mat.
	50	0	Scant.....	0	Heavy mat.
Acid Dox [2] solution with 1 c. c. of glycerin added to each flask.	900	0	Scant.....	0	Fair, white.
	500	0	Scant.....	0	Fair, white.
	125	0	Scant.....	0	Fair, white.
	50	0	None.....	0	Fair, white.
Alkaline Dox solution with 1 c. c. of glycerin added to each flask.	900	0	Very scant.....	0	Weak.
	500	0	Very scant.....	0	Weak.
	125	0	Very scant.....	0	Weak.
	50	0	None.....	0	Weak.
Raulin solution...	900	0	Fair.....	0	Heavy mat.
	500	0	Fair.....	0	Heavy mat.
	125	0	Fair.....	0	Heavy mat.
	50	0	Fair.....	0	Heavy mat.

[1] Raulin solution: 1,500 parts of water; 70 parts of cane sugar (35 gm. levulose); 4 parts of tartaric acid; 4 parts of ammonium nitrate; 0.6 part of ammonium phosphate; 0.4 part of magnesium carbonate; 0.6 part of potassium carbonate; 0.25 part of ammonium sulphate; 0.07 part of zinc sulphate; 0.07 part of iron sulphate; 0.07 part of potassium silicate.

[2] Dox solution, etc. (Czapek): Distilled water (H_2O), 3,000 c. c.; magnesium sulphate ($MgSO_4$), 1.5 gm.; dibasic potassium phosphate (K_2HPO_4), 3.0 gm.; sodium chlorid (KCl), 1.5 gm.; ferrous sulphate ($FeSO_4$), 0.03 gm.; with Potassium acid phosphate (KH_2PO_4), acid solution (Thom, 1910).

TABLE X.—*Effect of aeration: Tests with various nutrient solutions*—Continued

Solution.	Size of flask.	Sealed.		Check.	
		Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
	C. c.				
Raulin solution + ½ c. c. *M/1* calcium nitrate ($Ca(NO_3)_2$).	900	0	Fair, mat.....	10	Fair, thin mat.
	500	0	Fair, no mat.....	10	Fair, thin mat.
	125	0	Fair.....	25+	Mat.
	50	0	Fair.....	25+	Mat.
Raulin solution with levulose, on filter paper cones.	900	0	Filter covered....	0	Paper covered.
	500	0	Filter covered....	0	Paper covered.
	125	0	Less than above...	0	Paper covered.
	50	0	As above.....	0	Paper covered.
Acid Dox solution + 1 c. c. *M/10* arabinose.	900	5	Scanty white.....	25+	Fair, white.
	500	1–2	Scanty white.....	25+	Fair, white.
	125	0	Scanty white.....	50+	Fair, mat.
	50	0	Scanty white.....	50+	Fair, mat.
Acid Dox solution + 1 gm. potato starch.	900	?	Strong.....	20+	Strong, mat.
	500	?	Strong.....	20+	Strong, mat.
	125	0	Fair.....	20+	Strong, mat.
	50	0	Fair.....	20+	Strong, mat.

This experiment shows the effect of scanty aeration in repression of growth as well as an almost complete suppression of pycnidia in the sealed flasks. In the two cases where pycnidium production did take place in the sealed flasks, the fructification occurred in the larger flasks of the series. It must be said that the check flasks, especially the larger sized ones, were almost dry at the close of the experiment and the humidity conditions as well as the concentration were different from those of the sealed flasks. For the first three weeks, however, the cultures were approximately the same, and it seems safe to attribute the difference in growth and pycnidium suppression to improper aeration, rather than to the drying or concentration, especially since, as will be seen from later experiments, these factors play but little part in pycnidium production.

From the many observations recorded here, and from the experiments, it seems safe to conclude that this organism is very sensitive to the oxygen supply, and it requires good aeration for optimum growth and for pycnidium production.

HUMIDITY (TRANSPIRATION)

From a number of indications in cultures, it was felt that transpiration might be a factor of more or less importance in the growth and reproduction of this fungus. A study of the literature dealing with reproduction, especially the work of Klebs (1898) with *Sporodinia grandis*, made this

seem extremely probable. It was seen that cultures on various complex media did not produce pycnidia until they began to dry out, as a general rule. Moreover, on nutrient solutions the pycnidia commonly form on the surface. On vegetables, such as carrot or parsnip, or on prune-juice agar, the pycnidia formed in the aerial mycelium.

Very early this relation was suspected as being operative, and the filter paper cone was used in the first experiments to further transpiration and aeration. When, however, the relation was tested, it was seen that the actual formative influence of transpiration had been greatly overestimated. Filter-paper cones were compared with similar-sized disks of filter paper entirely submerged. Inoculation was made with bits of mycelium, and the cultures stood on the table in strong diffuse light.

TABLE XI.—*Effect of humidity: Test with filter paper*

[Time, 1 month]

Conditions.	Number of pycnidia.	Growth.
Cones mostly above water	5–10	Fair.
Submerged paper	10+	Scanty.

It is seen that the pycnidia production goes on after this period as strongly, if not better, in the submerged condition, while the growth seems slightly stronger on the cone. Since differences of this sort are hard to estimate, little importance is attached to the slight differences. Nevertheless, we have in this experiment striking evidence that under conditions where transpiration is reduced to the zero point pycnidium production is nevertheless vigorous.

In this experiment the possible relation to contact stimuli is not avoided. The following observation is even more conclusive, for here contact relations are limited to the effect of mutual contact of the threads of the mycelium itself, and no further elimination of a hypothetical contact relation is possible. Several water blanks of ordinary distilled water were heavily inoculated with spores and mycelium, respectively. After a month the following observation was recorded:

TABLE XII.—*Effect of humidity: Test with inoculated water*

Form of inoculation.	Number of pycnidia.	Growth.
Spores	4–10	Fair amount of white, byssoid mycelium. Total submergence.
Mycelium	2–10	Fair amount of white, cottony mycelium. Total submergence.

From these experiments there can be little doubt that pycnidia can be produced by this fungus without reference to the factor of transpiration.

We now come to an experiment in which the time element was recorded and in which the influence of a number of different degrees of air humidity was tested.

Four bell jars with a hole in the top were connected with a compressed-air reservoir so that a gentle current of air could be sent through the apparatus. The air was led into the bell jars by a tube reaching to the bottom of the bell jar and taken out by short tubes which extended through the stopper but a short distance. To secure moist conditions the air was bubbled through distilled water, while dry air was obtained by sending the blast through two towers filled with calcium chlorid. The first bell jar received moist air constantly, the fourth dry air constantly, and the second and third were connected by Y tubes to both the dry and wet bell jars, so that they could be made to receive either wet or dry air independently. Throughout the experiment the conditions in these two bell jars were alternated. The second bell jar received wet air for three days and then dry air for one day, while the conditions were reversed for the third jar. Preliminary tests with a Lamprecht polymeter in each jar (these were set to agree with a sling psychrometer reading) showed that the humidity within the first jar ranged from 65 per cent to 70 per cent, and in the fourth the humidity was only 20 per cent. In the other bell jars a humidity of 65 per cent or a dryness corresponding to 25 per cent could be obtained in a half hour by blowing in wet or dry air. The blast was almost continuous throughout the experiment except for a period each day between about 3 a. m. and 8 a. m., at which time the pressure was lacking. The bell jars giving wet conditions were fogged at times, but, as the apparatus was in strong light and as the fog disappeared except when the bell jars were hit by a cold draft, it is very likely that the light intensities were sufficient in all cases. For media various substances were used. Bits of pear and apple twigs, corn meal, slices of carrot and apple, peas, rice, and corn, as well as corn-meal agar and glucose agar, were autoclaved. The media were prepared in capsules without the covers and were placed in tiers in round wire baskets so that each capsule had free access to air. The basket was slipped inside a battery jar and was covered with a cotton pad held in place by a glass plate. Five sets of this sort were prepared, four to be subsequently placed under the bell jars, and the fifth to be used as a check without aeration. The media were autoclaved and then inoculated with a drop of spore suspension to each dish. The cultures were left one week under ordinary room conditions. At the beginning of the test of the various air conditions the bell jars were drenched with solution of mercury bichlorid. The basket was lifted under aseptic precautions and set upon a small metal rack. This

rack, which had been previously disinfected, rested upon the ground-glass base. The bell jar was quickly put in place over the basket and sealed air-tight by the use of anhydrous lanolin. Since the air pressure at times amounted to several pounds, these bell jars had to be clamped to the base plate. This was accomplished by boards drilled at the corners, the top one fitted with a 3-inch hole, through which the top of the bell jar projected. Long bolts fitted with thumb screws held the boards in place and thus when tightened prevented the jars from leaking. The air was filtered through cotton before it reached the cultures. Several times during the experiment the cultures subjected to dry air were moistened with a few cubic centimeters of water. It was found that those cultures were nearly dried out at these times.

No pycnidia were formed with peas, rice, or glucose agar under any of the conditions. Other cultures showed the pycnidia in the same relative proportions for the various conditions of aeration. The record for corn broth may be cited as typical.

TABLE XIII.—*Effect of humidity: Test with corn broth under bell jars*

[Time, 30 days]

Medium.	Number of pycnidia.					Growth.				
	Unaerated.	Wet.	Mostly wet.	Mostly dry.	Dry.	Unaerated.	Wet.	Mostly wet.	Mostly dry.	Dry.
Corn broth.....	o	o	o	+	+++	+++	++++	++++	+++	++

Pycnidia had been formed for some time before the reading was made. The aeration was continued, and a month later another reading was made. At this time all the cultures except peas, rice, and glucose agar showed pycnidia, irrespective of the air condition, with the exception of the series left as a check. This series, left in a battery jar, covered with a cotton pad and a glass plate of the same size as the jar, made good growth, but in no case did pycnidia occur.

We have in this experiment results which indicate that at most the effect of moist air is to delay pycnidium formation. Whether this effect is due to decrease in transpiration or to nutrition conditions, either of the substratum or of the aerial mycelium, brought about by the excess of water in the air or condensed upon the hyphæ is not known, but it seems likely that the water relation is the most potent one, since with such efficient aeration the transpiration must be considerable in all cases. The previous experiments indicated that absence of transpiration was not directly inhibiting to pycnidium formation with cultures which were under conditions of scanty nutrition. The last experiment reiterates that conclusion, but indicates that the humidity may serve to delay fruit-body formation. The effect of moist air in delaying but

not suppressing pycnidium formation is always associated with increased aerial growth. When it is recalled that with rich media the pycnidia are commonly formed in the aerial mycelium, this opposed condition may be significant. Further discussion of this behavior is given at another place (page 741).

In conclusion, it may be pointed out that transpiration, or, better, low air humidity, is a factor of only secondary or contributing influence in fruit-body formation for this fungus, and in no sense is a positive determining factor like light or aeration.

Physicochemical Factors

Reaction of the Substratum

The acid or alkaline reaction of nearly all biological fluids—the blood, milk, sea water, cell sap—varies but slightly from neutral. It is commonly said that fungi grow best under slightly alkaline conditions. Many organisms show great tolerance to either alkalinity or acidity, but the organism here investigated showed a comparatively narrow range, and its optimum point was not that of the great group of fungi, but much more like the optimum for bacteria.

The following experiment with filter-paper cones and with Raulin solution shows something of the limits of growth and reproduction for this organism. The acidity or alkalinity [1] indicated in the table was obtained by the addition of either normal potassium hydroxid or hydrochloric acid (potassium hydroxid in case of the Raulin solution, since it was acid at the outset).

Table XIV.—*Effect of acidity and alkalinity: Test with Raulin solution and filter paper*

[Time, 1 month]

Reaction.	Raulin solution.		Filter paper.	
	Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
−10	.....	Contaminated	.....	None.
− 5	.....	None	.....	None.
0	+	Strong	20	Scant.
+ 5	++	Strong	20	Scant.
+15	o	Fair	.....	None.
+28	o	Fair	.....	None.

This experiment showed the strict relation of this organism to the chemical reaction, both as to growth and as to reproduction, and, as usual, the growth limits were wider than the limits of reproduction. The experiment also revealed why Raulin's solution had previously

[1] Computed in terms of cubic centimeters of normal hydrochloric acid or potassium hydroxid in a liter by titrating 5 c. c. with *N*/20 standards, phenolphthalein as indicator.

given growth but no fruiting bodies. Once this relation of the organism to acid and alkali was known, previous experiments could be reviewed in the light of it and the behavior of certain chemicals explained.

Ten c. c. of a 5 per cent gum-arabic solution was autoclaved in a series of preparation dishes. The solution received sterile chemicals to give concentration as shown in the table and was inoculated with a spore suspension.

TABLE XV.—*Effect of acidity and alkalinity: Test with various chemicals*

[Time, 1 month]

Chemicals.	Concentration.	Reaction.	Number of pycnidia.	Growth.
Gum-arabic solution plus—				
Potassium acid phosphate	*M*/200	+	++	++
Potassium acid phosphate + Sodium acid phosphate	*M*/200 each	+	+++	+++
Sodium acid phosphate	*M*/200	+	++	+++
Dibasic potassium phosphate	*M*/200	−	o	+++
Check		±	+	++
Sodium hydroxid		−5	o	+

This experiment, if it be permitted to draw conclusions by comparison of salts with a similar anion or cation, indicated that the specific effects in pycnidium formation were not due to any specific ion, for if potassium were the influential ion, then we should get no effects with the similar sodium salt. More conclusive still was the effect of the dipotassium phosphate as contrasted with the dihydrogen salt. Here the same ions were concerned, but in different proportions. The experiment shows the extreme sensitiveness of this organism to alkalinity, since a reaction of −5 was sufficient to cause absence of pycnidia.

A study of the reaction of some of the common media, as given in Table XXV, shows how reaction controls not only reproduction, but growth as well. Of the complex media tried the most favorable for pycnidium production was a couple of corn grains autoclaved in 10 c. c. of water. Aside from the nutrition relation, which will be discussed later, the acid reaction is largely responsible for the excellence of this medium; but the time when this reaction is most effective is at the period when growth has covered the medium, not the mere reaction at the start. Corn broth shows at the start an acidity of +8, and after a month the reaction is still acid, +5. As is seen from Table XIV, this is a favorable condition for pycnidium production. Pea solution at the beginning of a period of culture showed an acid reaction of +8, while oats showed at the start a reaction of +5. The latter showed after a month a reaction approximately neutral. It will be seen from Table XXV that oats were a correspondingly poorer medium than corn. Pea broth, on the other hand, showed a reversal of condition, and after a month

titrated −8. The culture grew vigorously for a week or two, formed a mat and some aerial mycelium, then the gradual checking of the growth occurred. The culture ceased producing aerial mycelium and the mat became half submerged. Soon all growth ceased and the culture grew but indifferently or not at all when transplanted.

If old pea-broth cultures were acidified to approximately +5 with potassium acid phosphate, tartaric acid, or hydrochloric acid, growth started again and pycnidium production took place upon the dense mat.

Other media showed similar changes in either acid or alkaline reaction, and, as a rule, it may be said that media with a proportion of protein lower than the carbohydrate proportion show after a period of growth an acid reaction (Wehmer, 1891). With media high in protein the reaction becomes alkaline (Nägeli, 1880).

The consideration of the acidity or alkalinity of substrata at the start and at the close of a period of culture leads naturally to a consideration of autointoxication. This is especially appropriate in this case, since the autointoxication effects observed were due to harmful reactions produced by the by-products of metabolism. These by-products were not of the complex type commonly thought of in connection with the term autointoxication, but were mostly the simple and well-known end products of carbon and nitrogen dissimilation. The injurious effects were produced to a large extent by the acidity or alkalinity engendered, and the same effects could be artificially produced in a favorable medium by mere change of reaction.

Depending upon the excess of carbohydrate or protein, as has been said, the reaction of the substratum became either acid or alkaline. In the case of excess of carbohydrate, oxalic acid is formed by this organism, and in old cultures of corn, oats, or prune-juice agar crystals of calcium oxalate were often found. In the case of protein excess, as was demonstrated for old pea-broth cultures, the medium contained an excess of ammonia. This ammonia could be detected by boiling the liquid from such old cultures and testing the fumes with a strip of wet, red litmus paper.

In a solution where the carbohydrates and protein constituents are present in a proper ratio, these by-products of metabolism neutralize each other. Corn broth is a notable example of this type of medium, for in it the by-products, even after two months, are not potent enough to interfere with reproduction.

The action of these autointoxication products in the substratum is further illustrated by the common experience met with in transferring from old cultures of this organism. In old agar cultures of various sorts the mycelium was found dead when it was submerged in the substratum, although the aerial mycelium remained alive for more than a year.

We have, therefore, in autointoxication a phase of the major factor, acid or alkaline reaction, and while definite harmful bodies of a protein or amid type are known for organisms and may have been present here, we have in the end products of protein and carbohydrate dissimilation harmful constituents whose influence may be to limit either growth or reproduction.

Chemical Factors

Quantity of food

The quantity, rather than the quality, of the food needed for this organism can more conveniently be considered at the outset. As was stated at the beginning of the experimental work, there is a certain minimum for growth and also for reproduction. Naturally, reactions taking place at the base level of nutrition are sharper and less obscured than those taking place where food is in abundance and the factors of reaction, autointoxication, etc., have greater and greater influence. For this reason, once the capacity of this organism to grow and reproduce upon material almost devoid of nutrients was recognized, many of the experiments with other factors have been performed with the food supply reduced to a low level.

This power to grow upon simple stuffs and with them in extremely high dilution naturally led to the question of the minimum essential. Growth and reproduction in distilled water has already been mentioned. The distilled water used in the first experiments was the ordinary distilled water of the laboratory. The glassware used was "resistance," cleaned as described. The test tubes were plugged with cotton, and a few motes of cotton could be seen upon the surface of the water after inoculation. Inoculation was made as described with a spore suspension. The number of colonies which resulted from inoculation with similar-sized drops of this suspension in Raulin solution was from 5 to 20. These details show that a very small amount of organic stuff was introduced from the inoculum. After three or four weeks a white or gray filmlike mycelium could be seen, either attached to the glass or floating near the bottom of the test tube. After a month or, at times, two months 2 to 5 pycnidia were produced under the water.

It is difficult to understand where the carbon and nitrogen used by the fungus came from. The minerals might be accounted for more or less satisfactorily by assuming that they came from the glass, which is slightly soluble. For the organic stuffs we have a few possibilities. The nitrogen may have come from ammonia in the air, and the carbon from the small bits of cotton dropped from the cotton plug. It is more than likely that the distilled water carried some oily volatile material, which, while not strongly influencing conductivity, gave a suitable foodstuff for the fungus. Or we have the possibility, first pointed out by Elfving (1890), that organisms may be fed by small quantities of volatile sub-

stances which are absorbed from laboratory air by the water (Beijerinck and Van Delden, 1903). Be the source of this food supply what it may, I was interested to find if all distilled water, even the purest, had enough food supply or absorbed enough to support both growth and reproduction.

Conductivity water[1] of a value 3.03 times 10^{-6} was used as in the preceding experiment, with, however, the following improvements in the method. Jena glass test tubes were used throughout. The test tubes were plugged with long-fiber absorbent cotton, and the preliminary dry sterilization, which has a tendency to make the fibers brittle, was omitted. Inoculation was made with one drop of a filtered spore suspension which had about 25 to 50 spores to the drop. The pycnidium which furnished these spores was growing in aerial mycelium, so none of the old substratum was brought over. At all events, material brought with the spores was diluted nearly 200 times. After two months slight growth was evident as faint submerged wisps or skeins. The growth was less than a tenth as strong as that produced in ordinary distilled water. No pycnidia were formed.

This experiment indicates that in the soluble glass and in the character of the distilled water we have the important sources of the food supply. The motes of cotton were practically eliminated in the last experiment. It might be thought that the nutrition in this case was as good as the preceding—assuming the food supply to come from volatile chemicals—and that the poor growth of mycelium and the failure to reproduce was due to the toxicity of the conductivity water. But the toxicity of ordinary distilled water is generally admitted to be greater than the toxicity of conductivity water. Moreover, this organism has never shown any effects which might be attributed to toxic substances in the water. In the recent experiments on the toxicity of distilled water with other plants the nutrition phase has been neglected, since the conclusions have been drawn from tests with the well-nourished roots of seedlings. In the experiments here reported, the food supply carried in the plants is that which is within a few spores barely visible with the high power of the microscope. It is difficult therefore to attribute the effects to anything but the scantiness of nutrition.

The conclusion, therefore, is drawn that while growth and reproduction can take place with the meager food supply of ordinary distilled water in "resistance" glass, the limit of reproduction is reached with conductivity water and Jena glass, but the limit of growth is still lower.

This same relation to nutrition was shown with the following experiment with filter paper. It had been determined in many previous experiments that this organism could grow and reproduce upon filter paper and distilled water. Tests with tap water, distilled water, and conductivity water indicated that the material used for growth and

[1] I am indebted to Dr. R. P. Hibbard, of the Michigan Agricultural College, for the conductivity water. The measurements of resistance were also made by him.

reproduction came largely from filter paper. Although filter paper is said to be the purest form of cellulose obtainable, Schwalbe (1910–11, p. 600) states that appreciable amounts of oxycellulose and hydrate-cellulose are present. Since filter paper is known to have some ash, a preliminary experiment was performed to find if this ash served, in part at least, as a source of food. A pair of culture dishes was prepared with a filter-paper cone in each. Ten c. c. of ordinary distilled water were added. To each of two other dishes with a similar amount of water, the ash from a filter cone was added. These dishes were autoclaved. Inoculations were made with spores. After three weeks the results shown in Table XVI were obtained.

TABLE XVI.—*Effect of quantity of food: Test with filter paper and the ash from filter paper*

[Time, 3 weeks]

Medium.	Pycnidia.	Growth.
10 c. c. distilled water, plus filter cone	+	Good.
10 c. c. distilled water, plus ash	−	Scanty.

The better growth and the pycnidial production on the filter paper, as opposed to the results with ash, indicate that the influential stuffs are not those from the ash. It may be remarked that the readings were taken early enough to avoid complications due to the slow pycnidium formation in distilled water. The effect of ash having been shown to be negligible, the main experiment was set up. Five sheets of filter paper (S. & S. 595) about 15 cm. across were autoclaved in 500 c. c. of conductivity water in a Jena flask. This furnished a stock solution, which was diluted with conductivity water by means of pipettes and graduates, which were carefully rinsed before and during the operations. The dilutions were prepared in Jena beakers, but were eventually put in 10 c. c. quantities in a number of Jena test tubes. These were autoclaved and inoculated with a spore suspension. This experiment was done in duplicate with each of the strains of the fungus, with the results shown in Table XVII.

TABLE XVII.—*Effect of quantity of food: Test with filter-paper broth*

[Time, 2 months]

Medium.	Pycnidia.	Growth.
Filter-paper broth:		
1/1	+ (3)	Fair, easily seen.
1/100	+ (1)	Fair, easily seen.
1/1,000	+ (1)	Scant, barely visible.
1/10,000	−	Scant, barely visible.
Conductivity-water check	−	Scant, barely visible.

The experiment shows that the ordinary high-grade filter paper, when autoclaved with water of high purity, yields sufficient nutriment for growth and reproduction of this organism. A dilution of 1/100 is still sufficient for pycnidium production, but at 1/1,000 we have reached the limit of food supply sufficient for pycnidium production. Growth, as usual, takes place at greater limits than reproduction.

This experiment gives conclusive evidence that the toxic substances of distilled water do not affect this organism. We may now conclude that we have been working nearer and nearer the limits of growth and reproduction. The amount of material required is evidently extremely minute. It is in the imponderable mass of stuff, somewhere between distilled water and conductivity water, or in that bulk of stuff lying between 1/1,000 and 1/10,000 dilution of a filter-paper broth.

Having now some conception of the extremely low limits of concentration at which growth may take place, we may now consider the growth and reproduction relations at higher concentrations.

The experiments already reported give a mass of details as to growth, at various concentrations, but no conclusions from these isolated cases are justified, because the reaction is so masked by other relations.

The following experiments allow a comparison of some nutrient solutions at various concentrations. The solutions chosen were those which did not become toxic with the continued growth of the organism. In one experiment 200 grains of corn were autoclaved in 1 liter of tap water. This solution was concentrated to approximately 100 c. c. by boiling in a beaker. It was, therefore, approximately 10 times the strength of ordinary corn broth. The strong solution was also diluted as shown in the table. Cultures were made as usual and were inoculated with a spore suspension. The results are shown in Table XVIII.

TABLE XVIII.—*Effect of quantity of food: Test with corn solution*

[Time, 1 month]

Concentration.	Pycnidia.	Growth.	Remarks.
10×	−	+++++	White.
5×	−	++++	White.
1×	+++	++	Blackened.
1/10×	+	+	Blackened.

In this experiment it is seen that the organism, after a month, produced fruiting bodies only in the lower concentrations, but the growth was strong in the higher concentrations. The growth in the weaker concentrations had increased but slightly after the first two weeks. We may conclude then that a food supply which allows a fair growth and then becomes exhausted is most favorable for pycnidium formation.

The following experiment with synthetic media was performed. The combination described upon page 752 was made up at 25 times the usual

concentration. This was diluted as shown in Table XIX, and cultures were made as in the preceding experiment.

TABLE XIX.—*Effect of quantity of food: Test with synthetic solution*

[After 1 month]

Dilution.	Pycnidia.	Growth.	Remarks.
25×.....	—	o	
10×.....	—	o	
5×.....	o	++++	White or pinkish.
2×.....	o	+++	Black mat formed.
1×.....	Many.	+++	Slightly less growth than above, black mat.
1/2×.....	Many immature.	++	Slightly less growth than in 2×. Abundant evidence of pycnidia starting.
1/5×.....	10	+	Growth weak.
1/10×....	5	+	Pycnidia extremely minute. Mycelium scanty.

The experience with this solution shows that doubling the concentration of a favorable culture solution increased growth, and was sufficient to inhibit completely pycnidium formation. A solution diluted one-half gave promise of many pycnidia—more than in the 1× concentration—but the pycnidia were slow in forming. In the extremely low concentration growth was scant and a small amount of pycnidium production took place. The experiment leads to the same conclusions as the preceding experiment—i. e., that a limited food supply is essential to fruit-body formation, and the optimum concentration is one which gives a comparatively large mycelial growth before the exhaustion takes place.

The teaching of this experiment would place the limit of concentration of a sugar at *M/100*. We have, however, a great body of experiments already outlined in which pycnidium production took place with a sugar concentration considerably higher. For instance, in Table X pycnidia are reported for Raulin's solution (cane sugar *M/7*) when a calcium salt was added. Or, considering the experiments with corn grains, these seem to present a contradiction when it is noted that the pycnidia were first formed on the corn grain with its rich food supply. Similarly, the various laboratory media—such as prune-juice agar, parsnips, and carrots—all are rich in carbohydrates; yet these are reported as allowing pycnidium production.

In these rich solutions, however, an extremely abundant aerial mycelium is produced, and as the medium begins to dry the pycnidia are produced in the aerial strands, but never upon the medium itself. In a few cases a dense mat formed over the agar, and this effectively walled off the new food supply. On only one laboratory medium—corn-meal agar (Shear and Wood, 1913)—were the pycnidia produced directly upon the agar. It is noteworthy that with this medium the mycelium production is scant. In the case of corn grains the pycnidium production does not take place until the corn grain is dried somewhat, and this, coupled with

the fact that the corn grain is not extremely soluble, accounts very well for the appearance here. Instead of the corn grain furnishing nutrition, the corn grain soon becomes the location where food supply is soonest exhausted. In this behavior upon drying, we may also find the explanation of the behavior of the wet and dry bell jars reported in Table XIII. The behavior of the 1 × and ½ × concentrations of the synthetic medium may be considered in this connection. It seems that in this case we have a similar factor to deal with. The mycelium in these concentrations grows at the top of the solutions, a trifle submerged in the case of the weaker solution. The stronger mycelial growth in the higher concentration leads to the formation of a thicker surface film in it than in this weaker one, and the film starts much sooner. The pycnidia are produced upon this surface film, which, no doubt, in some ways interferes with the utilization of the food supply.

From this it would seem that the limiting concentration suggested—*M/100* for sugar—instead of being too low is doubtless too high, and the production of pycnidia at this concentration, at the period stated, is brought about by the other factors, which lead to an even greater reduction of the available concentration.

When we consider the action of this aerial life of the mycelium in fostering reproduction, we find that our knowledge of the transfer of materials in mycelium is extremely limited. It, however, seems very likely that with the increase in concentration in the medium below and the drying of the threads, the diffusion of foodstuffs to the aerial parts is interfered with.

QUALITY OF FOOD

MINERALS.—The work with the quantity of foodstuffs just outlined indicates the extreme difficulty of determining what minerals are essential for growth. This sensitiveness to extremely small amounts, which doubtless is paralleled by other organisms, makes experimentation with ordinary methods or ordinary chemicals unreliable. The problem of determining the necessary mineral elements for this fungus would be impossible with our present technic.

An attempt was made to find the effect of certain chemicals when they were added to various nutrient solutions. Although many experiments were performed, the results were so masked or influenced by the constituents of the medium that no conclusions could be drawn. Notable influences which have been explained as other than nutrition effects have been obtained with acid phosphates and with calcium compounds.

The behavior of one chemical, magnesium sulphate ($MgSO_4$), is worthy of record. Since Molisch's accurate work (1894), this substance has generally been regarded as essential in fungous cultures. The following experiment suggests that the chemical may have a profound effect upon fructification. Two preparation dishes each received 10 c. c. of a solution

containing magnesium sulphate in *M/33* concentration. Conductivity water was used. Inoculation was made with a drop of spore suspension. After one month many (more than 50) pycnidia were found in the loose submerged mycelium.

As a mineral base for nutrient solutions, monobasic potassium phosphate and magnesium sulphate, along with other chemicals, were frequently employed. The net result of numerous cultures made in the attempt to find some hint of the value of this or that mineral was the conclusion that cultures with these two constituents alone, with a suitable nitrogen and carbon supply, gave as good results as more complex combinations.

This solution of mineral salts contains the bulk of the elements generally considered essential for fungus growth. Carbon and nitrogen need to be added to secure the complete nutrient, but iron can be neglected, since it is such an unavoidable impurity in chemicals and is usually present as a constituent of the glassware. Beijerinck (Samkow, 1903) had used a similar solution as a culture medium for bacteria.[1]

Because of the extremely small amounts of minerals found necessary for growth and reproduction in this form, I modified the formula by cutting down the concentration of the various components. Since the solutions were to be used in comparative work, the chemicals were added on a molecular-weight basis. At the time of the first experimentation it was thought that the reaction should be approximately neutral, and accordingly molecularly equivalent weights of potassium acid phosphate and sodium carbonate were employed. Similarly, through dependence upon relations of other plants, it was thought that magnesium sulphate might be slightly toxic, and it was used at a lower concentration than either of the other two minerals. The solution thus devised for preliminary experiments contained sodium carbonate and potassium acid phosphate as *M/100* and magnesium sulphate as *M/500*. Subsequent experiment showed that the carbonate could well be omitted and the magnesium sulphate increased from fivefold to tenfold.

The other combinations were used for comparison with this mineral base. The mineral constituents of Raulin solution and those of Dox solution were tried, and while either were suitable, neither had any advantage over this modified Beijerinck solution; on the contrary, they were much more complex and contained the mineral elements in excess of the needs of this fungus.

CARBON SUPPLY.—The carbohydrates form the common source of carbon for fungi. Other classes of compounds, as pointed out by Nägeli (1880) and Wehmer (1891), may be utilized. For this organism, as indicated in Table XXIII, other classes of compounds—but of alcoholic

[1] Samkow used the following base with a great variety of organic compounds: Potassium acid phosphate, 2 gm.; sodium carbonate, 2.5 gm.; magnesium sulphate, 0.4 gm.; water, 1 liter.

structure—may be utilized as a carbon source (malic acid and glycerol). As is well known, various plants possess widely varying amounts of sugars, and the sugars and other carbohydrates differ markedly in kind. The specific effects of certain vegetable media have been attributed by many to the specific action of the type of carbohydrate furnished. Roux and Linossier (1890), as a result of their work with the fungus *Dematium albicans* Laurent, announced as a general biological law that with an increase in the molecular weight of the carbohydrates the complexity of the growth form of the fungus increased. With certain sugars, such as glucose in a 1 per cent solution, these investigators obtained only yeastlike growth, but with a biose, such as maltose, they obtained strong mycelium and conidia production. Recently Hiekel (1906), repeating the work of Roux and Linossier, but with 10 per cent sugar solutions, accepted the conclusions of the French investigators within certain limits. A priori, it is very difficult to see why two sugars, such as glucose and maltose, should differ in specific effects, since the latter, when hydrolized, yields only the former.

Very early in the investigation tests were made with the common sugars to find whether there was a specific effect on fruit-body formation due to the various sugars. In these tests the sugars used were used as weight-normal solutions; hence, the effects secured were not obscured by concentration differences. The various sugars were added from a sterile stock *M/1* solution to 10 c. c. of the autoclaved nutrient solutions, as indicated in the table. Glass preparation dishes were used, and all were placed in strong diffuse light. Inoculations were made with spore suspension in the usual manner. The tests were done in duplicate. Table XX shows the average of conditions.

TABLE XX.—*Effect of quality of food: Test with sugars*

Sugar.	Pea broth.			Oat broth.			Tap water and filter.		
	Sugar concentration.	Pycnidia.	Growth.	Sugar concentration.	Pycnidia.	Growth	Sugar concentration.	Pycnidia.	Growth
Saccharose	M/10	o	+++	M/10	o	+++	.....	.....	.....
Do	M/20	o	++++	M/20	o	+++	.....	.....	.....
Do	M/50	o	+++	M/50	o	+++	M/50	o	++
Dextrose	M/10	o	.....	M/10	o	+++	.....	.....	.....
Do	M/20	o	++++	M/20	o	+++	.....	.....	.....
Do	M/50	o	+++	M/50	o	+++	M/50	o	++
Levulose	M/10	o	+++	.....	.....	.....	.....	.....	.....
Do	M/20	o	++++	.....	.....	.....	.....	.....	.....
Do	M/50	o	+++	.....	.....	.....	M/50	+ (1 or 2)	+
Maltose	M/10	o	++++	.....	.....	.....	.....	.....	.....
Do	M/20	o	++++	.....	.....	.....	.....	.....	.....
Do	M/50	o	+++	.....	.....	.....	.....	.....	.....
Check	.....	o	++	.....	+	++	M/50	o	++
							.....	+	+

It will be noticed that in nearly every case, even in low concentration of sugar, there was an increased growth following the addition of sugar. Filter paper and oat broth, which normally produce pycnidia, gave strong growth with saccharose, dextrose, and maltose, but no pycnidia. In the case of levulose *M/50*, the growth was not greatly increased, and one or two pycnidia appeared. This number is much less than the normal for filter paper alone. We may conclude that these sugars exert a repressing influence on pycnidium production, and at the same time augment vegetative growth. How this is brought about is difficult to explain; but in some way the ratio of the constituents was so altered that the limits for reproduction of some factor—e. g., reaction—or of some group of factors was exceeded.

A more comprehensive experiment was performed in which a large number of carbohydrates was tested. Equal parts of the minerals of Raulin's solution in 2× concentration were added to various *M/10* sugar solutions and to 2 per cent solutions of the polyoses whose molecular weight is not known. Each combination was set up in four capsules, using 10 c. c. per dish. The media were steamed on three successive days and inoculated with a drop of spore suspension for each dish. Table XXI gives the result of this experiment.

TABLE XXI.—*Effect of quality of food: Test with carbohydrates*

[Time, 2 months]

Carbohydrate.	Concentration.	Size of colonies.	Growth.	
			Character.	Form of fructification.
		Mm.		
Xylose (pentose)	*M/20*	3–4	Compact	Oidia.
Maltose (disaccharose)	*M/20*	3	Compact	Oidia.
Glucose (monosaccharose)	*M/20*	2	Compact	Oidia.
Mannose (monosaccharose)	*M/20*	2	Compact	Oidia.
Galactose (monosaccharose)	*M/20*	2	Compact	Oidia.
Levulose (monosaccharose)	*M/20*	1–2	Compact	Oidia.
Arabinose (pentose)	*M/20*	1–2	Compact	Oidia.
Sorbose (monosaccharose)	*M/20*	(2½)	Floccose	Pycnidia.
Sucrose (disaccharose)	*M/20*	½–1	Compact	Oidia.
Raffinose (polysaccharose)	*M/20*	2–3	Floccose, very loose.	Mycelium.
Lichenin (polysaccharose)	1 per cent		Loose mat, covering dish.	Secondary spores.
Dextrin (polysaccharose)	1 per cent		Diffuse mat, covering dish.	Pycnidia.
Inulin (polysaccharose)	1 per cent		do	Pycnidia.
Gum arabic (polysaccharose)	1 per cent		do	Pycnidia.
Gum tragacanth (polysaccharose).	1 per cent		do	Pycnidia.
Wheat starch (polysaccharose).	1 per cent		No growth	
Lactose (disaccharose)	*M/20*		No growth	
Erythrose (tetrose)	*M/20*		No growth	

In the above table the sugars and other carbohydrates are arranged on the basis of vigor of vegetative growth. In the main the results of the former experiment are substantiated. The strongest growth took place with the highly soluble sugars, and the dishes were filled with small ball-like masses. The strongest growth was not associated with pycnidia production, but on the contrary was opposed to it. At first glance the law of Roux and Linossier (1890) seems operative, for pycnidia appeared in the carbohydrates, which are known to have extremely high molecular weights. But this superficial agreement is abundantly contradicted by the first part of the list. Without regard to molecular weight, these sugars gave approximately the same growth form, and the variation in amount of growth was not striking. It will be noted that these sugars are highly soluble, while those toward the bottom of the list are almost insoluble. In the one case every bit of the foodstuff was available, while in the other only a slight amount of the carbohydrate was open to appropriation. The preceding experiment with filter paper and sugars proved that, where the scant available carbohydrate of filter paper allowed pycnidia production, the addition of sugars destroyed the balance between growth and reproduction, and only growth took place. The same general relations exist between the members in this table as existed in the former experiment. It is worthy of note that Roux and Linossier (1890) and later Hiekel (1906) drew their conclusions from carbohydrates such as the first seven. We can find in their method of work the source of their error. Their solutions were made up on a percentage basis, and where they drew a conclusion that a complex sugar like maltose in 1 per cent solution gave a more complex growth than a 1 per cent glucose solution, because of the difference in molecular weight, they were in reality comparing *M/36* and *M/18* solutions, and their conclusion really applies to concentration. They had previously shown that a low concentration would call out more complex growth forms.

The cause of the variation in growth among the various sugars is not known. A great many factors undoubtedly enter. Nearly all the sugars used were split in approximately the same way by the various specific enzyms of the organism. Differences in absorption rates, in rapidity of enzymotic action, etc., may enter and be responsible for the differences in growth here recorded. It may further be remarked that although the sugars used were of the highest purity they vary in their relative freedom from contamination, owing to difficulties in separation and purification. The colloidal carbohydrates undoubtedly carry a mass of adsorbed material, while in the others, traces of calcium, nitrogenous material, etc., may be present. It is not unusual to find a minute gummy scum on freshly prepared maltose solution.

Certain other interesting points are to be found in the table. The production of the growth form called "oidia"—multiseptate, heavy-walled hyphæ resembling Dematium or at times Monilia—were constantly

found in the highly soluble sugars. Such growth forms have commonly been recognized as a reaction to high osmotic pressure. Ternetz (1900) has obtained these in acid solutions. But such growth forms have occurred with this fungus in distilled water and on filter paper, and no doubt this growth form, instead of being a specific reaction to concentration, is one induced by a number of unfavorable conditions.

The action of sorbose has been disregarded, because this sugar is broken down by heat. The failure to obtain growth with lactose and erythrose is not without parallel in the literature. The action of wheat starch is peculiar, in view of the previous successful use of potato starch (Table X).

The action of lichenin is of great interest. This carbohydrate is a dextrin-like compound, almost insoluble in cold water and forming a gummy mass in hot water. In the turbid solution of this chemical the fungus produced a great number of secondary spores, evidently hyphomycetous. These spores were of approximately the same size and shape as the ordinary spores of this fungus. The exact method of their production was not determined. Mounts of material gave only straight mycelial threads and great numbers of detached spores. Dilution plates poured from the culture dishes teeming with these spores gave no other organism than the one under investigation. The colonies appeared in the plates in such abundance as to leave no doubt concerning the relation of these colonies to the secondary spores.

The experiments with carbohydrates may now be summarized. Nearly all carbohydrates tried served as a source for carbon. The general effect of adding sugars even in so low a concentration as *M/50* was to stimulate vegetative growth greatly, but this stimulated growth was accompanied by a pronounced repression of pycnidium formation. In an experiment with *M/20* solutions a strong mycelial growth was obtained, accompanied by oidia-like bodies, but fructification was absent. With slightly soluble carbohydrates, in which the actual amount of available soluble material was always limited, vegetative growth was weaker and pycnidium production was a general rule. A comparison of these highly soluble and slightly soluble carbohydrates indicates that the differences in growth form are connected with the amount of food supply rather than with the specific nature of the sugar. This position is reinforced when we consider that the hydrolysis of inulin, gum arabic, etc., yields exactly those sugars which, when tested in *M/20* concentration, gave no pycnidia. In view of this comparison the earlier conclusion of Roux and Linossier (1890) seems untenable, and a more plausible explanation of the differences of growth form obtained seems to be found in the concentration relations.

This matter of carbohydrate supply has obviously a marked influence upon the problem of the organic media for laboratory use.

NITROGEN SUPPLY.—That the organism was influenced by the kind and amount of the nitrogen supplied seemed evident from the results of experiments with standard media, such as beef broth and beef agar, as well as the results already reported for pea broth.

A number of preliminary experiments of the same type as those reported under carbohydrates were performed at the same time, and these indicated that the various nitrates influenced pycnidium formation. But these results were not altogether consistent. The following experiment (see Table XXII) with filter paper and tap water plus various chemicals, and the similar series in which distilled water was used, may be cited as typical.

TABLE XXII.—*Effect of quality of food: Test with various nitrates*

[Time, 1 month]

Chemical.	Present as—	Distilled water.		Tap water.	
		Pycnidia.	Growth.	Pycnidia.	Growth.
Calcium nitrate	*M/100*	20–30	++	20–50	+++
Potassium nitrate	*M/100*	50–100	+++	50–100	+++
Calcium acid phosphate ($Ca(H_2PO_4)_2$) + calcium nitrate.	*M/200*, *M/100*	30–50	+++	100+	++
Potassium acid phosphate + potassium nitrate.	*M/100* each	.....	+++	100+	+
Filter paper	Check	9–20	+	7–12	+

From this experiment it could not be determined beyond question that the nitrate ion was the potent factor in this increase in pycnidia formation, but the corresponding behavior of both the calcium and the potassium nitrate indicated that this was extremely likely. The increase in pycnidium production upon the addition of both a phosphate and a nitrate to this carbohydrate medium is significant.

Since the nature of the carbon assimilation might greatly influence the nitrogen assimilation, experiments with these two compounds can hardly be separated. In the following experiment an attempt was made to test various classes of carbon-furnishing compounds with various nitrogen sources. In this experiment the mineral solution mentioned in the preceding section was used. The stock solution contained monobasic potassium phosphate as *M/100*, sodium carbonate as *M/100*, and magnesium sulphate as *M/500*. To different portions of this, malic acid, glycerol, and maltose were added, respectively, so that each chemical was present at *M/100* concentration. A fourth series was prepared as a check, and in this cones of filter paper furnished the carbon supply (S. & S. 605). The various solutions were put into series of preparation dishes, 5 c. c. per dish. To these dishes the nitrogen compounds to be tested were added from a clean pipette 1 drop (1/20 c. c.) of the proper solution (stock solutions were made up *M/50*, except peptone, which was 2 per cent) to

each dish. The various combinations employed, and the dilutions present in the culture, are indicated in Table XXIII. In every instance the concentration given shows the amount of the chemical that was present in the culture. The experiment was done in quadruplicate.

TABLE XXIII.—*Effect of quality of food: Test with nitrogen and carbon compounds*

Stock solution of minerals plus—		Number of pycnidia.	Growth.
Carbon.	Nitrogen.		
Malic acid, *M*/100	Peptone, 0.02 per cent	100	Scant.
Glycerol, *M*/100		60	Strong.
Maltose, *M*/100		0	Strong.
Filter paper		.....	No growth.
Malic acid, *M*/100	Asparagin, *M*/100	.....	None.
Glycerol, *M*/100		5	Scant.
Maltose, *M*/100		100+	Strong.
Filter paper		.....	None.
Malic acid, *M*/100	Leucin, *M*/600	5	Scant.
Glycerol, *M*/100		0	Scant.
Maltose, *M*/100		25–50	Strong.
Filter paper		.....	None.
Malic acid, *M*/100	Potassium nitrate, *M*/500	.....	None.
Glycerol, *M*/100		2–7	None.
Maltose, *M*/100		50	Fair.
Filter paper		0	Scant.
Malic acid, *M*/100	.....	10–20	Fair.
Glycerol, *M*/100	.....	0	Scant.
Maltose, *M*/100	.....	0	Fair.
Filter paper	.....	10–15	Fair.
Peptone	.....	1–5	Scant.
Asparagin, *M*/500	.....	0	Scant.
Leucin, *M*/600	.....	0	Scant.
Potassium nitrate, *M*/500	.....	0	Scant.

This experiment shows that nitrogen, as previously shown for carbon, may be taken from widely different classes of compounds. The availability of any particular nitrogen compound is largely determined by the associated carbon compound. For instance, peptone, which carries available carbon, gave a large number of pycnidia with malic acid, but none with maltose. Asparagin, which gives the best growth and the greatest number of pycnidia with maltose, gave no pycnidia with malic acid. Glycerol, which seems on the whole to be a poor carbon source, gave with peptone strong pycnidium production, but with other nitrogen compounds behaved indifferently. As a further complication, peptone is able to serve both as nitrogen and as carbon source. Leucin gave poor growth with all carbon compounds except maltose, and a comparison of its behavior with that of asparagin, which is a compound of the same class, is interesting.

The experiment shows in a striking way how unlimited the possible combinations of nutrients may be. The marvelous thing is the absolute regularity of the product, regardless of this or that varied food supply. Growth that morphologically could not be distinguished arose from a protein or a mineral nitrate. Pycnidia were produced from these widely divergent compounds, with carbon compounds equally separated, and in these were billions of spores which did not differ in a manner permitting measurement, each a potentiality which could repeat indefinitely under these conditions the same reaction.

From this experiment we may pick a combination which is favorable for growth, but which also gives an abundance of pycnidia. For further experiments the combination of minerals with maltose and asparagin was chosen. The steps, more or less logical, which lead to the development of this synthetic culture solution may be reviewed. Experiment had shown that the essential mineral elements necessary for the growth and development of this fungus were contained in two mineral salts. Experiments in which these are added to various nutrient solutions give a hint as to the value and the concentration suitable. Eventually a compound was selected which gave the mineral salts which needed to be supplied and in addition had a chemical which could be used to make the reaction less acid, as desired. Previous work had shown that the organism could grow and produce pycnidia on extremely limited amounts of minerals, so the amounts taken were extremely small—far smaller than the ordinary formulas call for. In choosing the carbohydrate, wide choice was possible, since so many allowed good growth. Maltose was selected for use in the experiment just reported, because, next to xylose, it had given the best growth. The use of xylose was not advisable because of its high cost, but care was taken to use maltose in small amount, so that the effect found in the experiment reported in Table XX would not be repeated. Accordingly, *M*/100 concentration was provisionally chosen. The device for deciding upon the nitrogen source has been detailed in the preceding experiment. The low concentration of nitrogen was chosen to avoid such toxic conditions as were found in the pea broth. In passing, it may be said that an attempt was made to secure approximately the ratio of carbon to nitrogen that exists in the corn broth, which had been found extremely favorable to the organism.

Different concentrations of the separate constituents of this nutrient solution were further tested, with extremely interesting results. The device used was to vary the concentration of one constituent while holding the others constant. It was thought that in this way approximately the optima for all the constituents could be found.

The following experiment was performed with double-distilled water (slightly poorer than conductivity grade) and "non-sol" glass flasks. Dilutions were prepared as outlined in the table, and the culture media

steamed on three successive days. It was found that steaming instead of sterilization under pressure was very important. In a previous attempt the media were sterilized in the autoclave, and upon inoculation absolutely no growth took place. The experiment was done in quadruplicate with one strain. Inoculation was made with a spore suspension as before. The flasks were set in strong diffuse light near a window. Readings were made after a month. Five c. c. of water were added to each flask, and a second set of readings [1] were made after another month. The result of the experiment is shown in Table XXIV.

TABLE XXIV.—*Effect of quality of food: Test with synthetic solution in various combinations*

[Time, 2 months]

Medium.			Number of pycnidia.	Growth.
Potassium acid phosphate, *M*/100. Sodium carbonate, *M*/100. Maltose, *M*/100. Magnesium sulphate, *M*/500	Plus asparagin	*M*/50 *M*/100 *M*/500 *M*/1,000 *M*/5,000	0 0 50 0 0	± ± ++ + +
Potassium acid phosphate, *M*/100. Sodium carbonate, *M*/100. Magnesium sulphate, *M*/500 Asparagin, *M*/500	Plus maltose	*M*/10 *M*/50 *M*/100 *M*/200 *M*/1,000	0 0 50 0 0	+++ +++ ++ + +
Potassium acid phosphate, *M*/100. Sodium carbonate, *M*/100. Maltose, *M*/100. Asparagin, *M*/500	Plus magnesium sulphate.	*M*/50 *M*/100 *M*/500 *M*/1,000 *M*/5,000	[a] 13 1 50 0 0	++ +++ ++ ++ +
Potassium acid phosphate, *M*/100. Magnesium sulphate, *M*/500 Maltose, *M*/100. Asparagin, *M*/500	Plus sodium carbonate.	*M*/10 *M*/50 *M*/100 *M*/200 *M*/1,000	0 0 50 90 [b] 200	o + ++ ++ +++
Magnesium sulphate, *M*/500 Sodium carbonate, *M*/100. Maltose, *M*/100. Asparagin, *M*/500	Plus potassium acid phosphate.	*M*/10 *M*/50 *M*/100 *M*/200 *M*/1,000	8 0 50 0 0	+ ++ ++ ± o

[a] 50 in 1.

[b] 295 in 1.

The device adopted is seen to be a very helpful one in determining the value of the various concentrations employed. The cultures in which asparagin was varied show how fortunate a concentration was chosen in the preliminary experiments. Similarly the experience with maltose shows that if asparagin is taken as *M*/500 then the maltose must have

[1] I am indebted to my colleague, Mr. J. H. Muncie, for making these readings.

approximately five times the strength. The experiments with magnesium sulphate are contradictory in part, but when the experience on page 742 is considered it may be concluded that for this organism the magnesium-sulphate ratio may be increased with profit. The phosphate proportion represented by *M/100* seems to be the favorable one. Sodium carbonate is found to be a constituent entirely unnecessary and for the most part detrimental to fruit-body formation.[1]

By this experiment, which could profitably be carried still farther within the limits indicated, a synthetic culture medium was obtained which gave for this organism a far greater pycnidia production than any other medium tried.

The merits of this medium may now be considered. It is a solution which contains the minerals necessary for growth of a vigorous character, but these chemicals are not present in superfluous amounts. It contains the carbohydrate which gave a remarkably strong, vigorous growth with this fungus, but the amount of the sugar is limited. The nitrogen source is a chemical of known composition and with maltose gave the strongest pycnidium production in the previous experiments. From the behavior of this organism we may conclude that we are approaching an ideal culture medium for the growth and reproduction of this organism. But we may go even farther, since the physiological relations of fungi to the substratum are so much alike. We can safely say that this combination will be found widely useful in producing similar reproduction in related forms. By the application of the same type of manipulation, some such combination can be found for other forms which will give better results than are now obtained on the ordinary media.

We may now consider some of the ordinary laboratory media in their effects upon this organism. The fungus has been cultivated upon a great many of the ordinary materials used in the laboratory for stock cultures and for diagnostic work. In this culture work the relation to light and to oxygen has been carefully observed. The relation to reaction has been but tardily recognized. The experience reported for pea broth shows that almost all relations to media can be reversed by changes in reaction (acidity or alkalinity). The initial relation is not, however, of as much importance as the reaction to phenophthalein after sufficient growth has taken place to lead to pycnidium formation. Table XXV summarizes the behavior of the organism on the complex media, with the relations on the synthetic solutions included for comparison.

[1] For convenience the amounts used in preparing this solution may be given. Stock solutions of *M/5* chemicals are prepared as follows:

Magnesium sulphate+7 Aq. 2.466 gm.+50 c. c. water.

Potassium acid phosphate 1.36 gm.+50 c. c. water.

Asparagin 1.33 gm.+50 c. c. water.

Maltose 3.60 gm.+50 c. c. water.

For 100 c. c. synthetic solution take 1 c. c. of *M/5* magnesium sulphate and 5 c. c. of each of the other solutions, and add to 84 c. c. water. Steam on three successive days.

TABLE XXV.—*Effect of quality of food: Complex media compared with synthetic solution*

Medium.	Reaction.		Growth character.	Aerial form.	Pycnidia.	Time.
	Start.	One month.				
Prune-juice agar (broth from 75 gr.).	+8	±	Strong white mycelium, becoming greenish. Medium reddened.	Strong tufted..	+++[a]	3 weeks.
Glucose agar[b] (glucose 3 per cent; peptone 1 per cent).	.....	.....	White, restricted growth becoming red-brown; oidia.	Prominent....	o	
Corn-meal agar (Shear)...	+10	+8	Weak growth of mycelium, mostly submerged; no mat.	Scant, if any..	++	4 weeks.
Standard agar.....	+15	−5	Strong growth, white, forming mat.	Scant, if any..	o	
Standard gelatin.....	+20	−5	Strong growth, white, gelatin slowly liquefied.	Fair amount..	o	
Filter paper.....	±	±	Scant amount creeping from point of inoculation, becoming greenish black, paper not discolored.	.....	++	1–3 weeks.
Parsnip plug.....	.....	.....	Strong, quickly covering plug, which shrivels. Color white, then tawny	Tufts.....	+++	4 weeks.
Carrot plug.....	.....	.....	As above, color greenish at close.	Tufts.....	+++	4 weeks.
Pea broth (2 seeds in 10 c. c.).	+8	−8	Strong, forming tough mat, which becomes submerged; white.	Scant.....	o	
Corn broth (2 grains in 10 c. c.).	+8	+5	Scant to medium amount, submerged, forming a film, otherwise no aerial growth, blackening at time of fruiting.	.....	++++	15 – 20 days.
Beans (2 seeds in 10 c. c.)..	.....	.....	As in peas.....	.....	o	
Bananas (autoclaved)....	.....	.....	Strong, covering slice, reddish brown when old.	Strong.....	+	Slowly formed.
Rice plus 5×water.....	.....	.....	Strong, covering grains, which blacken after a month; white mycelium becoming gray-green.	Strong tufted .	o	
Oats (2 grains in 10 c. c.).	+5	±	Weak, submerged growth forming film; blackens in a month.	.....	+	4 weeks.
Raulin solution.....	+30	+20	Strong white, becoming tawny.	Strong tufted .	o	
Synthetic solution: Potassium acid phosphate, *M*/100. Magnesium sulphate, *M*/500. Maltose, *M*/100..... Asparagin, *M*/500....	+30	+5	Good white growth, submerged, forming film on surface, on which pycnidia form; mycelium blackens just before fruiting.	.....	++++++	4–6 weeks.

[a] Aerial. [b] One formula calls for 200 gm. of glucose per liter (Harter, 1913).

A comparison of the media with reference to their reaction (+ or −) has already been made. In this relation we have a sharp determining factor which eliminates many preparations. Other media, such as rice, may be taken as cases where a poor balance exists between the nitrogen supply and the carbon supply, thus setting up an unfavorable toxic condition. The corn broth and the synthetic solution behave alike. The aerial growth seems to be strongest in substrata of an acid character. With rich substrata pycnidium production is aerial. The rapid production of pycnidia on filter paper is very significant. The wide range of suitable media is of great importance, and, since these substances must present carbohydrates and nitrogen compounds in great variety,

we have in these complex forms the same sort of result as was obtained in Table XXIII. But, in spite of the variety, the growth is much the same, and when fruiting bodies are produced they are the same morphologically. Such uniformity can be explained only by the assumption of an assimilation process which deals with much the same stuffs in all the substrata. The reserve materials are then worked over by the protoplasm under favorable conditions, and the fructification takes place.

Effect of Change of Intensity of a Factor during the Growing Period

Those experiments of Klebs (1899) in which a bit of rapidly growing mycelium of *Saprolegnia mixta* was transferred from a good nutrient solution to another of poorer quality, with resulting strong response in sporangium production, are the most striking demonstrations of the relation of checked growth to reproductive processes. In experiments of this type we have a device for studying some of the factors with the aim of their further simplification. We must, however, recognize that, no matter how ingeniously the term "checked growth'" fits the phenomena described, it really tells us little about the physiological processes underlying.

The following experiment was performed. Strongly growing mycelium (1 week old on corn broth) was washed in two changes of 500 c. c. each of conductivity water. This mycelium was cut in pieces approximately the same size with sterile scissors and was added to the various sterile solutions shown in the table, with the results shown in Table XXVI.

Table XXVI.—*Effect of change of intensity of a factor: Withdrawal of food supply*

[Time, 1 week]

Medium.	Number of pycnidia.	Growth increment.
1-week-old mycelium added to—		
Filter paper	25	++
Conductivity water	5	+
Corn broth, 1/40	25	++
Corn broth 1×	0	++++
Magnesium sulphate, approximately *M*/100	2	+
Pea broth	0	+++++
Pea broth, 1/40	0	++
Check (similar mycelium allowed to grow undisturbed)	0	++

It is evident from these results that with the withdrawal of the food supply from vigorous, susceptible mycelium reproduction sets in promptly. The results were obtained in one week—two weeks after inoculation—although normally pycnidium production with corn grains is much slower. This hastening of the reproductive process by change of quantity of food supply indicates that here we were able to produce the change which takes place more slowly in the ordinary cultures.

The following experiment (see Table XXVII), which was performed as part of the experiment given on page 741, gives the effect of change of concentration upon the mycelium. The experiment was made with corn broth and with synthetic solution. The transfer was made after three weeks' growth had taken place.

TABLE XXVII.—*Effect of change of intensity of a factor: Change of concentration of food supply*

CORN BROTH

Extent of change.	Number of pycnidia.	Growth increment.
From 10× to 10×	2	++
From 10× to 5×	25	+
From 10× to 1×	25	+
From 10× to 1/10×	25–40	+
From 5× to 10×	(*a*)	.....
From 5× to 5×	.....	.....
From 5× to 1×	2–25	++
From 5× to 1/10×	.....	.....
From 1× to 10×	50	++
From 1× to 5×	4–9	++
From 1× to 1×	0	+++
From 1× to 1/10×	12–15	+
From 1/10× to 10×	.....	.....
From 1/10× to 5×	0–3	++
From 1/10× to 1×	0	++
From 1/10× to 1/10×	.....	.....

SYNTHETIC SOLUTION

From 10× to 1×	0	+++
From 10× to ½×	100	++
From 10× to 1/5×	20	+
From 5× to 25×	0	++++
From 5× to 10×	0	++++
From 5× to 2×	0	++ *b*
From 5× to 1×	100	+++
From 5× to ½×	50	++
From 5× to 1/5×	50	++
From 5× to 1/10×	25	+
From 1× to 10×	0	+++++
From 1× to 5×	0	++++
From 1× to 2×	0	++++
From 1× to 1/5×	15	+
From 1× to 1/10×	12	+
From 1/5× to 5×	0	++
From 1/5× to 1×	100	+++
From 1/5× to 1/10×	0	+
From 1/10× to 2×	0	+++
From 1/10× to 1×	100	++
From 1/10× to ½×	50	++++
From 1/10× to 1/5×	50	+

a These transfers were not made. *b* Many immature.

The results given in Table XXVII show in striking manner the effect of the transfer of mycelium from one concentration to another. When mycelium from a poor solution is placed in a rich solution, it begins to grow vigorously, and, on the other hand, when rapidly growing mycelium is transferred to a solution of less concentration, the increase in growth is less. Exactly as the mycelium is checked or started into growth, reproduction is fostered or inhibited. While from the results of the experiments reported before it could only be said that these conditions of growth and reproduction occurred constantly side by side and therefore were related. From this last experiment we have definite proof of the interrelation of these two processes.

Other factors than food supply were experimented upon in the same way. The experiment previously reported under temperature (p. 726) strictly speaking belongs here. It may be remarked that pycnidium production began in the cold before it began in the cultures under room conditions. A similar experiment was performed with corn broth. Corn grains with mycelium about two weeks old, which showed no signs of pycnidium production, were set near a window at room temperature, and in the light in a cold attic where the temperature was about 10° C. After one week there were many pycnidia in the culture in the cold and the growth was checked, while in the culture under room conditions pycnidia production was just beginning and growth had continued regularly. After two weeks, however, the pycnidia were abundant in all the cultures, but were more abundant in the cultures under room conditions. From this experiment it is seen that a checking of growth by other means than food withdrawal can operate in much the same favorable way upon reproduction.

If, then, the factor light, which is known to have a strong power of checking growth, operates in influencing pycnidia production in this manner, we should be able to replace the light effect by checking the mycelial growth in some other way. Cultures, if left in the dark, ought to produce pycnidia eventually. Cultures with scanty food supply, such as those on filter paper, ought to yield pycnidia rather quickly in the dark. The experiments already reported have failed to show this action. Therefore, the action of light is not merely due to the checking influence which it has upon mycelial growth. If it were, we should have the paradoxical condition in which the withdrawal of light from a culture with limited food supply would augment pycnidium production, because of the greater growth in the dark and the more rapid diminution of the nourishment.

The following experiment (see Table XXVIII) was performed, in which the effect of checking the growth of corn-broth cultures by low temperatures was tried in both light and dark conditions. Corn-broth cultures 12 days old were placed under the conditions shown in the

table. The cultures in the dark were placed in the dark chambers described on page 723, and those in the light were placed in battery jars with tilted covers.

TABLE XXVIII.—*Effect of change of intensity of a factor: Change in temperature*

Conditions.	Pycnidia.		Growth increment, two weeks.
	One week.	Two weeks.	
Room temperature (22°):			
Dark	0	0	++++
Light	0–4	25–50	++
Approximately 10°:			
Dark	0	0	++
Light	10–15	10–25	++

The conditions were continued for two weeks longer without any change in the relations. This experiment reinforces the conclusion just arrived at that light has some other action than a mere checking of growth, and its action can not be replaced by a mere checking of growth.

Light is known to have a powerful oxidizing effect, and organic material under the influence of light is subjected, according to Freer and Novy (1903), to the action of organic peroxids engendered by the catalytic action.

The following experiment was tried to determine whether some such action was concerned. Hydrogen peroxid was added to 12-day-old corn-broth cultures at the rate of 2 drops (1/20 c. c.) of a 3 per cent solution to a dish. The experiment was checked with cultures of the same age. The dishes were placed in a dark chamber. After a week (first examination) the result shown in Table XXIX was obtained.

TABLE XXIX.—*Effect of change of intensity of a factor: Addition of hydrogen peroxid to corn broth*

Medium.	Pycnidia.
Corn broth + hydrogen peroxid (H_2O_2)	+[a]
Corn broth, check	−

[a] 4 to 8.

By strongly oxidizing cultures with hydrogen peroxid it was possible to replace the morphogenic action of light. Light, therefore, must act in some such manner upon this organism, and the action in fruit-body formation must be of some such character. This experiment was repeated at least six times, with varying concentrations of hydrogen peroxid. With cultures grown in the dark for from two to three weeks, the addition

of from 1/25 to 1/5 c. c. of hydrogen peroxid (3 per cent) would produce a few pycnidia with darkened cultures. In the stronger concentrations the mycelium was completely enveloped with a froth. After the first stimulation the cultures produced no further pycnidia. It must be said that in no case were pycnidia produced in amounts equal to those under light conditions. At best the use of hydrogen peroxid is a very harsh method.

With young cultures or with very old cultures the hydrogen peroxid was ineffective. In these its behavior is like that of light.

Other chemicals known to be strong oxidizing agents were employed. It may be said that nearly all gave positive results at extremely weak dilutions, provided that the mycelium used was in proper condition. Mycelium which would produce pycnidia by an hour's exposure to light gave good results with the oxidizing agents.

Another factor was doubtless responsible for the inequality of pycnidium formation in these experiments. All the chemicals used are toxic to the mycelium. In the concentrations used, these poisoned the cultures and certainly affected the reactions.

Table XXX summarizes the successful trials.

TABLE XXX.—*Effect of change of intensity of a factor: Use of various chemicals*

Chemical and concentration.	Corn broth.	Synthetic.	Pea.
Nitric acid (HNO_3), *M/500*	+	+	+
Sulphuric acid (H_2SO_4), *M/500*	+	+	−
Sulphuric acid (H_2SO_4), *M/500*, + potassium dichromate ($K_2Cr_2O_7$), *M/500*	+	+	−
Potassium permanganate ($K_2Mn_2O_7$), *M/500*	−	−	−
Ferric chlorid ($FeCl_3$), 1 drop of *M/5*	+	+	−
Zinc sulphate ($ZnSO_4$), *M/500*	−	−	−

GENERAL DISCUSSION

The work reported in this paper has given more or less of a definition of the environment in which *Plenodomus fuscomaculans* can live and reproduce. We now know the bare essentials for growth—the base level of existence—since we know the minima of the various formal conditions of growth. Similarly, we know some of the highest intensities which can be tolerated.

For growth at the base level of existence, there is only required the almost immeasurably small food supply of conductivity water, a scanty amount of free oxygen, and a temperature of 6° C.—perhaps lower. These factors may be increased in intensity until there is tolerated a food supply enormously larger, abundant oxygen, and temperatures up to 37° C.—perhaps higher. But as the simple minimum conditions are passed, the interactions of the component factors of the environment increase, and new factors arise which also have their limits. With

increase of food supply we must now consider, besides the mere chemical parts, the ratio of these parts to each other, both at the outset of growth and throughout the growing period. We analyze such relations and classify them as reaction, etc.

For pycnidium production the limits are found to be much narrower than those suitable for growth. No reproduction takes place at the base level of existence. Food supply must be increased, not greatly, but in measurable amount. From the scanty supply in conductivity water it must increase to the quantity found in distilled water—a two-fold to tenfold increase. Or it must be present in at least one thousandth of the quantity cooked from a few sheets of finest filter paper by conductivity water, but one-tenth of this amount is not sufficient. Oxygen must be present in abundance; stagnant air prevents reproduction. The temperature may be as low as 10° C., but must not be as low as 6½° C.

Up to a certain limit (perhaps up to $M/50$), increase in concentration of the food supply augments reproduction. After that point the excess food supply retards and eventually inhibits reproduction. Fructification, when it does take place with media of higher concentration, takes place in the aerial mycelium, and doubtless here the conditions are comparable to those in which the fructification is produced within or upon the medium.

The kind of food may vary almost without limit. An organism which can grow and reproduce in distilled water or a grain of corn can find requisite food materials in almost any biological product. But the more complex substances bring new relations, which, while of some importance to growth, are of decisive importance for reproduction. Growth can take place between the acid and alkali limits of $+30$ and -10 to phenolphthalein, but reproduction is limited to the conditions but slightly more acid than the neutral point of this indicator.

Corn broth seems at first glance a better foodstuff for this organism than oat broth, and in two parallel cultures the first will produce 50 pycnidia while the other is producing one. Yet if the oat culture be acidified with an acid phosphate, or even with hydrochloric acid, it becomes nearly as good a culture medium as the corn. Glucose agar made after the ordinary formula gives a strong growth with this organism, but no pycnidia. If the chemicals of this formula be diluted 50 times, the organism will fruit abundantly upon it. This organism was found to be greatly overfed by the ordinary laboratory media, and under the influence of the great excess of food grew and grew until the by-products of metabolism checked growth or destroyed the organism.

The differences in media were not so much in the food which they contained—for an examination of published analyses will show all necessary elements for growth and reproduction in almost any plant—as in the acid or alkaline reaction which the medium gave when prepared, the reaction maintained, and the concentration or relative scantiness of carbohydrate

and protein. The least adapted synthetic solution for this fungus (Raulin, 1869), could be made to yield pycnidia by the addition of lime, which probably counteracted the acidity; and pea cultures in which the mycelium was submerged and nearly dead could be made to grow and produce pycnidia by mere acidification. Furthermore, pea cultures to which sugar is added to balance the protein produce abundant pycnidia in the aerial hyphæ.

A consideration of the various laboratory media shows them to be rather purposeless, clumsy devices, in which this organism is overfed. Except the very simplest ones, none have warrant for existence if considered from the point of view of adaptability for a specific purpose. The great similarity of results on the various media seems to require the conclusion that these foodstuffs are not specific. Any fruit or vegetable is a full nutrient for almost any organism if the material be made properly soluble, and any harmful acid or alkaline reaction or otherwise unfavorable concentration be adjusted. Probably any biological product can likewise be utilized. Our methods have made a fetish of variety and have completely neglected the contributing factors.

As has been said, fungi behave alike in their relations to the substrata in the vast majority of cases. That the findings for this organism apply to others seems entirely probable. In many ways confirmatory evidence is to be found in the present practices. A certain medium is discovered which gives fruiting bodies for some fungus. A number of other organisms not at all related, in spite of differences in life relation, are also found to fruit upon this medium.

A consideration of one of the best preparations devised for fruit-body formation is very interesting. Shear's corn-meal agar is made by stiffening with agar the infusion obtained from four teaspoonfuls of corn meal (Shear and Wood, 1913). This medium is suitable for fructification for this organism, because it gives a scanty food supply, yet sufficient readily available to produce the growth necessary for pycnidium formation. The ratio of carbohydrate to protein is such that the reaction remains acid. Reasoning from such similar phenomena, a rather general application may be made. Any organism of this type can be made to grow and fruit upon a synthetic substratum containing the essential components, provided that the ratio of the components, hence the acid or alkaline reaction, and the concentration, be adjusted to the limits demanded by the particular organism. This assumes that the factors of light, temperature, aeration, etc., also fall within their own suitable limits.

We have, therefore, within the reach of experimental work the possibility of developing an environment which can be so defined that it can always be duplicated, suitable for a great group of organisms (Thom, 1910). With such a chemically and physically defined environment the classification of organisms could be placed upon a sounder working basis.

It is commonly admitted that the description of an organism must be taken under the assumption of some definite environment. The great mass of media in common use, the uncertainty of composition, the lack of standardization, and the usual failure to bring about fructification have left the description of fungi with only the natural habitat as a fixed environment. With forms of comparatively simple morphology this standard has led to the classification by hosts, with its attendant multiplicity of species. A firm basis for taxonomy can be arrived at, and simplification can come, only from a standardized environment.

As has been indicated in the preceding discussion, the physical environment must also be defined. With the growth of our knowledge of the forms we shall be able to a great extent to analyze the complex of forces. In the present paper one such force has been emphasized and its action discovered to be related to the liberation of energy by oxidation.

Light was found to be essential for reproduction. If light be absent or insufficient, although all other requirements were satisfied—with a medium suitable for growth and food supply, aeration, acid reaction, temperature, all within the proper limits—pycnidium production will not take place. Instead, aerial mycelium is formed, and eventually the organism goes into a static condition. The light factor, as others, has its limits. Weak light will not allow pycnidium production. This factor differs from the others in that its action need not be continuous. It is therefore of direct stimulative nature. A short exposure to strong diffuse light of cultures from dark conditions, which are otherwise ready for pycnidia formation, gives the necessary stimulus during a further period in the dark. When the effect of the stimulation is spent in the production of a few pycnidia, a second exposure is necessary for a second inauguration of the process.

The action of light in thus unlocking these forces is very satisfactorily explained by the experiment in which a few drops of hydrogen peroxid were used to replace the light stimulus. Other oxidizing agents also serve to stimulate fruit-body formation. The protoplasm of well-nourished mycelium is rich in oily reserve materials, and the action of light may oxidize these bodies and change them from emulsions of poor mobility to materials of great diffusibility. Accompanying this we have a releasing of energy, and fruit-body formation is inaugurated. The mechanism of this process is not known at all, but Herzog (1903) has shown that the sporulation of yeast is affected by temperature, and the curve for the variation in amount produced by temperature is a typical enzym curve.

Hydrogen peroxid added to a pea-broth culture, to a rich sugar solution, or to a young growing culture on corn broth does not immediately lead to fruit-body formation, nor does the action of light on such cultures lead to it. The action of light is modified and controlled by the condi-

tion of the mycelium, and this we have seen is a resultant of the environmental factors. In other words, we must consider in what way a mass of mycelium with checked vegetative growth is influenced to reproduction, while one in active growth is unaffected.

The cause of this relation to light, or, better, to oxidation, is understood if we take into account the fact that among organisms and among parts of the same organism there exists a strong competition for oxygen. In the cell itself the various processes inhibit and influence each other by their oxygen relations. Oxidation is never at its maximum in the cell under ordinary conditions, as simple tests with increased oxygen tensions show (Porodko, 1904). Organisms well aerated grow better than those in an air supply below the optimum. The action of oxidation is to release energy. The materials oxidized are either the foodstuffs suitable for nutrition or the cell material which growth has stored up. Euler (1909) contrasts growth, a stretching process, with reproduction, a differentiating and formative process. Growth is a process which is gradual, and it takes place even if only a small amount of energy be available. It is a process taking less energy than reproduction, as all respiration experiments have shown. The great consumption of energy in reproduction is doubtless associated with the great amount of nuclear protoplasm which must be formed. Growth, therefore, is the process first inaugurated, and the one which continues so long as the food supply is abundant and outer conditions permit. It is a static condition, as reproduction is dynamic.

In the hunger state the oxidations are different to a marked degree, as Kosinski (1901) has discovered, and here we have the cell reserve gradually drawn upon. The fats and even the proteins may be oxidized, according to Purievich (1900). But in this hunger state the respiration is reduced, according to Kosinski; hence, the working is slow. These metabolic relations, in spite of their great complexity, balance each other.

It would seem that reproduction is not possible under conditions favoring growth, because the oxygen supply is all used in ordinary metabolism. With the hunger state, respiration is reduced. Oxidation becomes vigorous if it be stimulated by light. No doubt any catalytic agent would be similarly effective. Once in this hunger state, oxidation, if augmented, takes place upon the rich cell stuffs, with the liberation of much energy. This energy is used in reshaping the reserve stuffs into complex protein bodies—the spores. The sharper the hunger condition is made, the more striking the reaction in pycnidium production. The sudden withdrawal of the food supply by the transfer of richly-growing mycelium to lower concentrations or to distilled water, checks ordinary assimilation, with its attendant use of oxygen. If oxidation of the cell reserves be inaugurated by light or some strong oxidizing agent, fructification takes place.

We may now consider other factors in the light of this theory. Experiment has shown that aeration is essential for reproduction. The action of light upon the protoplasm is dependent upon the oxygen supply. Aeration may work to continue the oxidizing process by the removal of end products, thus allowing oxidations to proceed to completion. In many cases recorded in the literature the effect of transpiration is to further the exchange of gases. The action of low temperature was to check growth, and pycnidium production was found to start. Euler (1909) states that lowering the temperature affects the oxidation process to a lesser degree than it affects other processes.

The action of the acid reaction is interesting and confirmatory. So far in this discussion the mechanics of the oxidation have not been considered. Oxidations in plants are generally believed to take place through the activity of oxidases of various sorts. As is well known, light activates this type of enzym, although it is detrimental to such enzyms as diastase (Euler, 1909, p. 97). The pronounced and sudden blackening of cultures about to produce pycnidia is very significant and can be best explained by the oxidation of some leuco compound by an oxidase (Kruse, 1910, p. 787). Some oxidases are known which work better in a slightly acid medium. We have seen that for this organism an alkaline medium was prejudicial to reproduction. The effect of acid reaction in favoring the reproductive process has not been explained, but it may have some connection with the enzymotic process. At any rate, an oxidation of oily stuffs to fatty acids would give a medium suitable for further activity of these ferments.

The formation of pycnidia in the aerial mycelium and in fact the whole series of complex reactions which Klebs (1900) has associated with "*Luft leben*" become much more comprehensible if we view them from the point of view of oxidation.

The replacement of the light factor by hydrogen peroxid thus becomes of great importance in reducing to simple terms the phenomena encountered. Light can unlock in suitable mycelium the reproductive process. This it does by its catalytic influence. The action may be due to the activation of oxidases along with the inauguration of a reaction (acid) favorable to their continued action; but this oxidation thus set up does not proceed to reproduction if the growth process is consuming the energy. If growth is not able to proceed, owing to scanty food supply or some checking influence, then the catalytic action of light inaugurates a building of the stored foodstuffs into complex fruiting bodies.

This general discussion may now be summarized. In the historical portion of the paper it was seen that the environment may be viewed as a directive and collective force which can be utilized for unfolding the life history of an organism. The great generalizations of Klebs are broad, and by their very broadness make possible acceptance in a wide

range of cases. Their teachings can not, however, be made the basis for research without the development of methods of attack suitable to a series of forms. The method of this paper may be used for similar organisms.

The first part of the paper may be interpreted as a determination of the limits of the life processes, which, when once determined, allow in the latter part of the paper a manipulation of them. The knowledge of the factors and their optima made possible a development of an environment especially fitted for growth and reproduction.

The proposition of Klebs, that the limits of reproduction are narrower than those of growth, is fully substantiated. Klebs further pointed out that growth and reproduction are processes opposed to each other. This is true for the organism studied.

The action of light has led to an insight into the mechanism of this opposed action. It has shown that growth, the static condition, is opposed to reproduction, a dynamic condition. Where one process is storing energy, the other is a process consuming energy. The equilibrium within the cells needs to be upset by some oxidizing force in the case of this fungus to inaugurate fruit-body formation in susceptible mycelium.

It is not concluded from the experiments with this species that light is a specific factor which will cause reproduction to take place in all forms, once growth is checked, although it may be expected to be an important condition in related organisms. But, in view of the great similarity of behavior in all the forms tested so far with respect to growth and reproduction, it may be concluded that in them some stimulus becomes operative when an organism is in the hunger state which starts the utilization by oxidation of the stored food supply and leads to the phenomenon of reproduction.

SUMMARY

This paper gives the results of experiments performed with *Plenodomus fuscomaculans*, a fungus pathogenic to the apple. The specific problem undertaken was the determination of the effects of various controlled environmental factors upon the growth and reproduction of this fungus.

The historical development of the art of culturing organisms has been traced from the first crude cultures to the present elaborate technic. The simultaneous development of our knowledge of the physiology of organisms has been briefly summarized. This survey shows that the environmental factors may greatly influence the life processes of organisms. Organisms have been cultured in the laboratory in an imitative or haphazard way, with a chance of finding a suitable environment. Owing to the great variety of available methods and the great plasticity of organisms, this course has been productive of results with some forms. Another type of research has sought to find the relation of the organism

to its environment and by manipulation of the environmental factors to discover the various phases of life history. Although many related forms have been grown in pure culture, very little physiological work of this type has been done with the Sphaeropsidales.

The organism was found to have a wider range of conditions suitable for growth than for reproduction. The base level of conditions necessary for growth is found in conductivity water at low temperatures. Reproduction requires more favorable conditions. Pycnidium production took place only in cultures exposed to light. The ordinary room temperatures were sufficient. Abundant aeration is essential. Transpiration is a factor of secondary importance. A slight acid reaction, especially at the close of the growing period, is a necessary condition. The value of a medium depends largely upon the acid or alkaline reactions present, not alone at the beginning but at the close of the growing period. Autointoxication was observed and was traced to excess of either acid or ammonia, which was the product of too great a proportion of either carbohydrate or protein, respectively.

As has been said, the quantity of foodstuff necessary for growth is extremely minute. Pycnidium production requires more food, but the meager amount present in distilled water is sufficient to allow the production of a few pycnidia. On the other hand, the fungus is able to tolerate very rich food supplies, but pycnidium production in solutions is restricted to $M/100$ or perhaps $M/50$ sugar concentration. Exact limits are hard to determine, because of the formation of mats or films in solutions, which effectively wall off much of the food supply. Fructification in the case of rich media takes place in the aerial hyphæ, and no doubt this relation corresponds with the conditions in solutions.

Magnesium sulphate and potassium dihydrogen phosphate in very dilute solutions furnish the necessary mineral elements for growth and reproduction. The carbon supply may be taken from a wide range of compounds of alcoholic structure. The carbohydrates furnish food materials in most available form, and, of these, xylose and maltose produce the best growth. The carbohydrates do not seem to be specific in producing fruiting bodies, and almost any are suitable if taken at the right dilution. The nitrogen assimilation is greatly influenced by the type of carbon nutrition.

The minerals mentioned and maltose and asparagin at the ratio of 5 to 1 seem to offer the most favorable combination, although others are suitable. From the experiments a medium was selected which though of entirely known composition gave better growth than any other tried. This synthetic solution had a scant amount of food supply, yet enough to permit a quick, vigorous growth. It retains the acid reaction till the close of the growing period. A study of this medium gave a basis for a criticism of results obtained with the common laboratory combinations.

The problem of this paper was a study of the effect of environmental factors upon this organism, especially as they influenced growth and reproduction. The experiments here reported verify the conclusions of Klebs and extend them for an untested group of organisms, the Sphaeropsidales. As has been pointed out, in this paper the method of approach was different from the inductive methods used by Klebs in drawing his conclusions, since the methods employed here were deductive, based on our knowledge of the reactions of other organisms. The experiments with *Plenodomus fuscomaculans* give a method applicable to related forms. The results of this physiological work give a basis for practical recommendations as to the culture of other organisms, as well as evidence of the feasibility of developing a standard synthetic solution which would make possible a standardization of environments for diagnostic purposes.

The action of light, when pushed to a last analysis and when considered in view of the experiment in which hydrogen peroxid and other oxidizing agents replaced it, is seen to be of either an oxidizing or a catalytic type. This led to the development of a theory to explain the mechanism of the opposed action of growth and reproduction. This theory sees in the competition for oxygen the fundamental reason for the absence of fructification under conditions which allow abundant growth.

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EFFECT OF ELEMENTAL SULPHUR AND OF CALCIUM SULPHATE ON CERTAIN OF THE HIGHER AND LOWER FORMS OF PLANT LIFE [1]

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INTRODUCTION

A study of the literature [2] shows that a number of investigators have noted a beneficial effect when elemental sulphur or sulphates are added to certain soils. The number of these investigations and also the types of soil and plants employed are limited. Certain workers report no beneficial effects from the addition of sulphur or sulphates to soil, and in isolated cases an injurious effect has been noted. Just how the sulphur or its compounds act is little understood, but there are two plausible explanations: (1) That it acts as a fertilizer, supplying the sulphur needed for plant growth, and (2) that it acts as a corrective agent—i. e., it favors beneficial groups of bacteria, while injurious forms are retarded in growth. However, the problem of sulphur and sulphates in agriculture is still far from being solved. This is especially true in the case of the effect of sulphur and sulphur compounds upon micro-organisms. In order to study this phase of the problem, a series of experiments was planned.

PLAN OF WORK

The object of these experiments was (1) to note the effect of sulphur and sulphates upon the soil micro-organisms and on pure cultures of legume bacteria, and (2) to note the effect of sulphur and sulphates upon the growth of red clover (*Trifolium pratense*).

For the experiments with mixed cultures, fresh soil was used as an inoculum. For legume bacteria all materials were sterilized, and the nutrient medium was inoculated with a pure culture of bacteria from the nodules of red clover.

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[1] Paper from the Laboratories of Agricultural Bacteriology and Agricultural Chemistry of the University of Wisconsin.

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Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bs

Vol. V, No. 16
Jan. 17, 1916
Wis.—3

EFFECT OF ELEMENTAL SULPHUR AND SULPHATES ON SOIL BACTERIA

MIXED CULTURES

For these experiments ten 1-gallon jars containing 2 kgm. each of Miami silt loam taken from the Wisconsin Experiment Station farm were used. The analysis of this soil is as follows:

	Per cent.
Potassium	2.16
Nitrogen	.15
Phosphorus	.15
Sulphur	.016
Calcium carbonate	.33
Humus	1.38

The moisture content of the soil was held at 18 per cent, or about half-saturation. Each jar was covered with a layer of cotton and gauze to prevent contamination, and was incubated at 28° C. Various amounts of sulphur and of calcium sulphate were added to the pots, as shown in Table I. At definite intervals samples were taken from the jars and bacterial counts as well as determinations of ammonia and of nitrates made. The results of the latter are given in Table I.

TABLE I.—*Effect of calcium sulphate and elemental sulphur on soil bacteria*

Treatment.	Number of organisms per gram of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated	6,866,000	8,746,000	10,790,000	6,350,000	10,782,000
Given 0.01 per cent of calcium sulphate	6,866,000	10,544,000	13,900,000	6,590,000	11,026,000
.05 per cent of calcium sulphate	8,506,000	14,140,000	13,789,000	8,028,000	9,945,000
.10 per cent of calcium sulphate	7,221,000	7,923,000	13,060,000	7,923,000	9,824,000
.50 per cent of calcium sulphate	8,290,000	8,029,000	13,420,000	7,548,000	10,305,000
1.00 per cent of calcium sulphate	8,580,000	9,585,000	12,938,000	7,668,000	9,945,000
Untreated	6,590,000	9,166,000	8,626,000	6,949,000	9,705,000
Given 0.01 per cent of sulphur	7,429,000	8,746,000	8,866,000	7,923,000	8,626,000
.05 per cent of sulphur	9,106,000	8,866,000	10,065,000	7,668,000	8,116,000
.10 per cent of sulphur	8,290,000	8,208,000	10,300,000	6,590,000	8,866,000
.50 per cent of sulphur	8,864,000	11,020,000	4,914,000	3,594,000	2,995,000
1.00 per cent of sulphur	6,504,000	7,070,000	2,635,000	2,329,000	719,000

The data show that calcium sulphate in the quantities used apparently has little effect on the number of soil organisms. Elemental sulphur, however, decreases the number of soil organisms that grow on agar plates. This decrease is not noticed until after 44 days, and only in soils to which 0.05 and 1 per cent of sulphur had been added. Quantitative acidity tests of the soils of these two jars showed it to be distinctly acid. This is corroborated by the work of Lint,[1] who has shown that in soil elemental sulphur is oxidized to sulphate and that the acidity produced is proportional to the amount of sulphur added. Acidity determinations were made according to Truog's[2] method and are given in Table II.

[1] Lint, H. C. The influence of sulphur on soil acidity. *In* Jour. Indus. and Engin. Chem., v. 6, no. 9, p. 747–748. 1914.

[2] Truog, E. A new test for soil acidity. Wis. Agr. Exp. Sta. Bul. 249, 16 p., 3 fig., 1 pl. 1915.

TABLE II.—*Acidity of soil treated with elemental sulphur*

Treatment.	Calcium oxid necessary to neutralize acid in 10 gm. of soil.
	Gm.
Untreated	0. 0000
Given 0.01 per cent of sulphur.	. 0000
.05 per cent of sulphur.	. 0000
.10 per cent of sulphur.	. 0011
.50 per cent of sulphur.	. 0369
1.00 per cent of sulphur.	. 0668

The results of these determinations show that the acidity produced by the oxidation of elemental sulphur to sulphate is proportional to the amount of sulphur added. In the samples to which 0.01 and 0.05 per cent of sulphur had been added, the soil contained enough lime to neutralize the acidity.

Change in reaction is probably the cause of the decrease in the number of the soil organisms. Abundant mold growth was found on the surface of the acid soils.

Table III shows that calcium sulphate in the quantities used has no effect on the production of ammonia in the soil. Elemental sulphur, however, in concentrations of 0.5 and 1 per cent increases the production of ammonia to a marked degree. This increase is noticeable after 44 days.

TABLE III.—*Effect of calcium sulphate and elemental sulphur on the production of ammonia in the soil*

Treatment.	Quantity (in milligrams) of ammonia nitrogen per 100 gm. of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated	3.99	3.19	3.40	3.21	3.23
Given 0.01 per cent of calcium sulphate	3.19	2.38	3.32	2.80	3.48
.05 per cent of calcium sulphate	3.19	2.21	3.06	3.06	3.57
.10 per cent of calcium sulphate	3.82	2.38	3.23	3.32	3.23
.50 per cent of calcium sulphate	3.19	2.29	3.36	3.40	3.57
1.00 per cent of calcium sulphate	3.82	2.21	3.06	3.23	3.40
Untreated	3.97	3.19	3.12	2.97	3.40
Given 0.01 per cent of sulphur	3.91	2.38	3.19	2.72	3.23
.05 per cent of sulphur	3.19	2.29	3.06	2.46	3.23
.10 per cent of sulphur	3.06	2.21	3.23	2.55	3.16
.50 per cent of sulphur	3.82	2.89	5.95	7.31	8.26
1.00 per cent of sulphur	3.95	2.89	6.80	7.31	9.52

The data in Table IV show that calcium sulphate in the quantities used does not materially affect the formation of nitrates in the soil. Elemental sulphur, on the other hand, in concentrations of 0.5 and 1 per cent decreases nitrate formation. This decrease is noticeable after 30 days. Previous to this time the sulphur does not seem to injure nitrate formation. Concentrations of sulphur lower than 0.5 per cent have no

appreciable effect on nitrification. It should be noted that while the bacterial counts begin to decrease after 44 days, the ammonia content begins to increase at this time.

TABLE IV.—*Effect of calcium sulphate and elemental sulphur on nitrate production in the soil*

Treatment.	Quantity (in milligrams) of ammonia nitrogen per 100 gm. of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated	1.87	1.34	2.95	3.93	4.70
Given 0.01 per cent of calcium sulphate	2.12	1.25	2.35	4.13	5.07
.05 per cent of calcium sulphate	2.42	1.01	2.49	4.58	5.72
.10 per cent of calcium sulphate	1.14	1.25	2.10	4.03	5.47
.50 per cent of calcium sulphate	1.86	1.23	2.45	2.92	4.99
1.00 per cent of calcium sulphate	1.35	1.82	2.39	2.87	4.51
Untreated	1.53	1.66	2.93	3.93	4.35
Given 0.01 per cent of sulphur	1.80	1.99	2.65	3.13	4.13
.05 per cent of sulphur	2.17	1.88	2.29	3.20	4.53
.10 per cent of sulphur	1.38	1.69	2.92	4.13	4.93
.50 per cent of sulphur	1.24	.54	1.41	1.14	.89
1.00 per cent of sulphur	.94	.54	.64	.95	.85

PURE CULTURES

In order to determine the effect of calcium sulphate on pure cultures of legume bacteria (red clover), Ashby's solution, minus the sulphate, was used. To 100 c. c. portions of this solution in 10 large Erlenmeyer flasks were added 30 gm. of pure quartz sand and various amounts of calcium sulphate. The sand was used to aid in breaking up the aggregates of bacteria when samples were taken for counts. All cultures were incubated at 20° C., and at intervals of one and two weeks bacterial counts were made. The results of these counts are given in Table V.

TABLE V.—*Effect of calcium sulphate on the growth of red clover organisms in Ashby's solution*

Treatment.	Number of organisms per cubic centimeter of solution after—		
	0 day.	7 days.	14 days.
Untreated	30, 000	53, 000, 000	157, 000, 000
Given 0.01 per cent of calcium sulphate	30, 000	139, 000, 000	425, 000, 000
.02 per cent of calcium sulphate	30, 000	177, 000, 000	400, 000, 000
.05 per cent of calcium sulphate	30, 000	198, 000, 000	450, 000, 000
.10 per cent of calcium sulphate	30, 000	121, 000, 000	350, 000, 000

The data show that the numbers of bacteria that grow on Ashby's agar were increased by the addition of calcium sulphate. The increase is very marked after both 7 and 14 days. It should be noted that 0.01 per cent of calcium sulphate is apparently just as efficient in producing an increase in the number of bacteria as is 0.1 per cent. This seems to indicate that only a trace of calcium sulphate is needed to stimulate the legume bacteria.

This experiment was repeated, using soil solution in place of Ashby's solution. For this purpose 1 kgm. of Miami silt loam was placed in a large container, 1 liter of distilled water added, and the entire mass boiled for one hour. It was next filtered, and 0.05 gm. of dipotassium phosphate and 1 gm. of mannite were added. This was then put into ten 500 c. c. flasks and 30 gm. of quartz sand added. Various amounts of calcium sulphate were used. The flasks were sterilized, and when cool were inoculated with a pure culture of red-clover bacteria. All cultures were incubated at 23° C. At intervals of one, two, and three weeks bacterial counts were made. These results are given in Table VI.

TABLE VI.—*Effect of calcium sulphate on the growth of red-clover organisms in soil solution*

Treatment.	Number of organisms per cubic centimeter of solution after—			
	0 day.	7 days.	14 days.	36 days.
Untreated	180,000	63,000,000	145,000,000	146,000,000
Given 0.01 per cent of calcium sulphate	180,000	135,000,000	176,000,000	237,000,000
.02 per cent of calcium sulphate	180,000	125,000,000	178,000,000	244,000,000
.05 per cent of calcium sulphate	180,000	125,000,000	269,000,000	259,000,000
.10 per cent of calcium sulphate	180,000	138,000,000	185,500,000	262,000,000

From the data it is evident that the addition of calcium sulphate stimulates the growth of red-clover organisms in pure cultures to the extent of more than 100 per cent. The results of this test agree with those obtained in Ashby's solution—i. e., that small amounts of calcium sulphate are apparently as beneficial as larger amounts.

EFFECT OF SULPHUR AND SULPHATES ON HIGHER PLANTS IN ARTIFICIAL MEDIA

Various experiments were made with the view of determining the effect of calcium sulphate and sulphur upon the growth of clover and upon nodule formation. This was tested first in artificial media. The medium consisted of a soft synthetic agar prepared from 1 liter of tap water, 5 gm. of dipotassium phosphate, and 7 gm. of agar. This medium was sufficiently firm to support the seeds. Thirty c. c. of the melted agar plus various quantities of calcium sulphate were added to each of 50 test tubes. In order to reduce the individual variation between the plants, 10 parallel tubes were used. The tubes were sterilized, and then two seeds of red clover were planted in each. After inoculation the cultures were removed to the greenhouse. At the end of two weeks greater root development was noted in the calcium-sulphate test tubes than in the untreated ones. In the older plants the increase in root development became most marked. The tops, however, failed to show any difference in size. In the tubes to which 0.1 per cent of calcium sulphate had been added, the plants were slightly smaller than the

others. At the end of six weeks the plants were removed and the roots measured. There was a distinct difference in root development, as shown in Table VII. Plate LVI, figure 1, shows very plainly the decided differences in root development. The results indicate that the increase in root development is as great with only 0.01 per cent of calcium sulphate added as with larger amounts. The test tubes treated with calcium sulphate were chosen at random from the calcium-sulphate series. They appear lighter because of the suspension of small particles of the salt in the agar.

The results of this experiment show that calcium sulphate greatly increases root development. However, in concentrations as high as 0.1 per cent, growth is slightly retarded. The increase in root development may be of considerable importance, first, because it enables the plant to reach out over a greater area for nourishment, and second, because of the greater field, the plant will be able to withstand drought better and thrive on poorer soil. The increase in root development may be the cause of the increase in the yield of clover when calcium sulphate is added to the soil. This is in confirmation of the work of Hart and Tottingham.[1]

These results are given in Table VII, which represents the average of 10 test tubes for each concentration used.

TABLE VII.—*Effect of calcium sulphate on the growth of red clover*

Treatment.	Length of root.	Length of stem.
	Cm.	*Cm.*
Untreated	3.8	4.2
Given 0.01 per cent of calcium sulphate	5.1	4.19
.02 per cent of calcium sulphate	5.5	4.7
.05 per cent of calcium sulphate	5.01	4.6
.10 per cent of calcium sulphate	4.93	3.3

EFFECT OF SULPHUR AND CALCIUM SULPHATE UPON CLOVER GROWN IN VARIOUS TYPES OF SOILS

The effect of calcium sulphate upon clover grown on Miami silt-loam soil was tested. For this experiment ten 1-gallon jars were used. Four kgm. of Miami silt-loam soil and various amounts of calcium sulphate were added to each. The jars were kept in the greenhouse and the moisture content held at 18 per cent. Each jar was seeded with red clover and then inoculated with a pure culture of red-clover organisms. After two weeks the jars were thinned to 10 plants.

During the first few weeks there was no apparent difference in the size of the plants. At the end of seven weeks an increase in growth in jars 3 to 8, inclusive, was noted. In jars 9 and 10, to which 0.1 per cent of calcium sulphate had been added, there was a decrease in growth. Four

[1] Hart, E. B., and Tottingham, W. E. Op. cit.

representative plants were removed from each jar. The roots of the plants grown in the sulphate-treated soil were longer and more branched than those of the plants grown in the untreated soil. There was an apparent increase in the number of nodules grown in the sulphate-treated series, except in the case of plants grown on soil to which 0.1 per cent of calcium sulphate had been added. The number of nodules on the above plants were about the same as on the plants grown in untreated soil. It must be remembered that the plants grown in the soil containing 0.1 per cent of calcium sulphate were smaller and therefore would naturally contain fewer nodules than the larger plants. Plate LVI, figure 2, illustrates these effects. The plants in group A were taken from the untreated soil; B, from the soil to which 0.01 per cent of calcium sulphate had been added; C, from soil to which 0.02 per cent had been added; D, from soil to which 0.05 per cent had been added; and E, from soil to which 0.1 per cent of calcium sulphate had been added. Note the marked increase in root development in B, C, D, and even E, where the plants are the same size as those in group A; also note that group E, to which 0.1 per cent of calcium sulphate was added, and D, to which 0.05 per cent was added, show no greater growth than A, the untreated, while groups B and C show an increase in top as well as root. The illustration shows very distinctly the increase in length of root and also the decrease in the growth of the plant under high concentrations of calcium sulphate. It is apparent that the addition of 0.02 and 0.05 per cent of calcium sulphate gave the most beneficial results.

TABLE VIII.—*Effect of calcium sulphate on the growth of red clover in soil*

Treatment.	Number of nodules.	Average of group.	Length of root.	Average of group.
	Cm.	*Cm.*	*Cm.*	*Cm.*
Untreated	9	10	6.5	7.2
Do	12		8.5	
Do	8		6.8	
Do	10		7.0	
Given 0.01 per cent of calcium sulphate	29	31	8.0	9.6
Do	33		10.0	
Do	11		10.5	
Do	51		9.0	
Given 0.02 per cent of calcium sulphate	34	33	8.0	9.5
Do	17		9.6	
Do	48		12.0	
Do	32		8.5	
Given 0.05 per cent of calcium sulphate	45	29	9.0	9.2
Do	36		6.5	
Do	18		11.3	
Do	19		11.5	
Given 0.1 per cent of calcium sulphate	13	14	7.0	7.6
Do	11		7.5	
Do	12		8.5	
Do	11		7.5	

The data in Table VIII show that calcium sulphate does increase the growth of the clover within a certain concentration. In amounts between 0.02 and 0.05 per cent it appears to be most beneficial. The results also show that calcium sulphate increases the root development and the number of nodules.

The effect of calcium sulphate on clover grown on Sparta acid sand was tested. Six kgm. of the sand admixed with 1 gm. of dipotassium phosphate were placed in each of ten 1-gallon jars. The composition of the Sparta acid sand used was as follows:

	Per cent.
Potassium	1. 16
Nitrogen	.062
Phosphorus	.034
Organic matter	1. 51

The jars were kept in the greenhouse and the moisture content held at 18 per cent. Each jar was seeded to red clover and then inoculated with a pure culture of red-clover organisms. After two weeks the jars were thinned to 20 plants in each. The plants grew luxuriantly, but there was no apparent difference in size until the sixth week. In jars 7 and 8, to which 0.05 per cent of calcium sulphate had been added, the increase in growth was considerable, while in jars 9 and 10, to which 0.1 per cent of calcium sulphate had been added, there was no appreciable increase. The jars to which 0.01 and 0.02 per cent of calcium sulphate had been added showed an increase in growth, but this increase was less than in jars 7 and 8. The green and dry weights of the clover were taken. The average weights of the clover are given in Table IX.

TABLE IX.—*Effect of calcium sulphate on red clover grown in Sparta acid sand*

Treatment.	Weight of crop.	
	Green.	Dry.
	Gm.	*Gm.*
Untreated	110. 6	19. 4
Given .01 per cent of calcium sulphate	131. 1	21. 2
.02 per cent of calcium sulphate	146. 7	21. 7
.05 per cent of calcium sulphate	168. 5	24. 6
.10 per cent of calcium sulphate	145. 8	17. 5

These results show that calcium sulphate increases the growth of clover grown on Sparta acid sand. The increase, however, is confined to certain concentrations. The greatest increase was obtained at concentrations of 0.02 and 0.05 per cent.

EFFECT OF ELEMENTAL SULPHUR ON GROWTH OF RED CLOVER

For this experiment ten 1-gallon jars, each containing 6 kgm. of Miami silt-loam soil, were used. Various amounts of sulphur were added. The jars were kept in the greenhouse and the moisture content

held at 18 per cent. After four weeks these were seeded with red clover and inoculated with a pure culture of red-clover organisms. Two weeks later the number of plants was reduced to six per jar. There was no appreciable difference in the size of the plants until the fourth month. At this time those in the sulphur series showed an increase in growth. At the end of the fifth month this increase was more marked. The leaves of the plants in the jars to which 0.05 per cent of sulphur had been added were tinged with red at the edges. The stem also showed this red coloration, but to a lesser degree. At the end of the fifth month the tops were cut and weighed, green and dry, with the results shown in Table X.

TABLE X.—*Effect of elemental sulphur on the growth of red clover*

Treatment.	Weight of crop.	
	Green.	Dry.
	Gm.	*Gm.*
Untreated	25.3	6.25
Given .01 per cent of sulphur	32.6	6.90
.02 per cent of sulphur	29.4	6.75
.05 per cent of sulphur	30.8	6.80
.10 per cent of sulphur	34.0	7.00

The sulphur series showed a slight increase in yield. Several of the plants died, so that the number of plants in the various jars varied. The results therefore are not final. It seems safe, however, to say that sulphur increased slightly the yield of clover in Miami silt-loam soil. After the tops were cut the roots were carefully removed and washed. There was no apparent difference in the size or the number of nodules in the treated and the untreated series. All of the roots contained a great number of nodules.

SUMMARY

(1) Calcium sulphate, when added to a soil, apparently has no marked effect on the total number of bacteria that grow on agar plates; nor does it produce any marked increase in ammonification or nitrification. This confirms the observations of Fred and Hart.[1]

(2) Large amounts of elemental sulphur cause a decrease in the total number of bacteria that grow on agar plates, but produce an increase in ammonification at concentrations of 0.05 per cent. This increase in ammonia is accompanied by a parallel decrease in nitrate formation. The decrease is very probably due to the acidity or toxicity produced by the oxidation of sulphur.

[1] Fred, E. B., and Hart, E. B. The comparative effect of phosphates and sulphates on soil bacteria. Wis. Agr. Exp. Sta. Research Bul. 35, p. 35–66, 6 fig. 1915.

(3) Calcium sulphate stimulates the growth of pure cultures of red-clover bacteria in nutrient solutions and in soil extract. The increase is as great with 0.01 per cent as with 0.1 per cent.

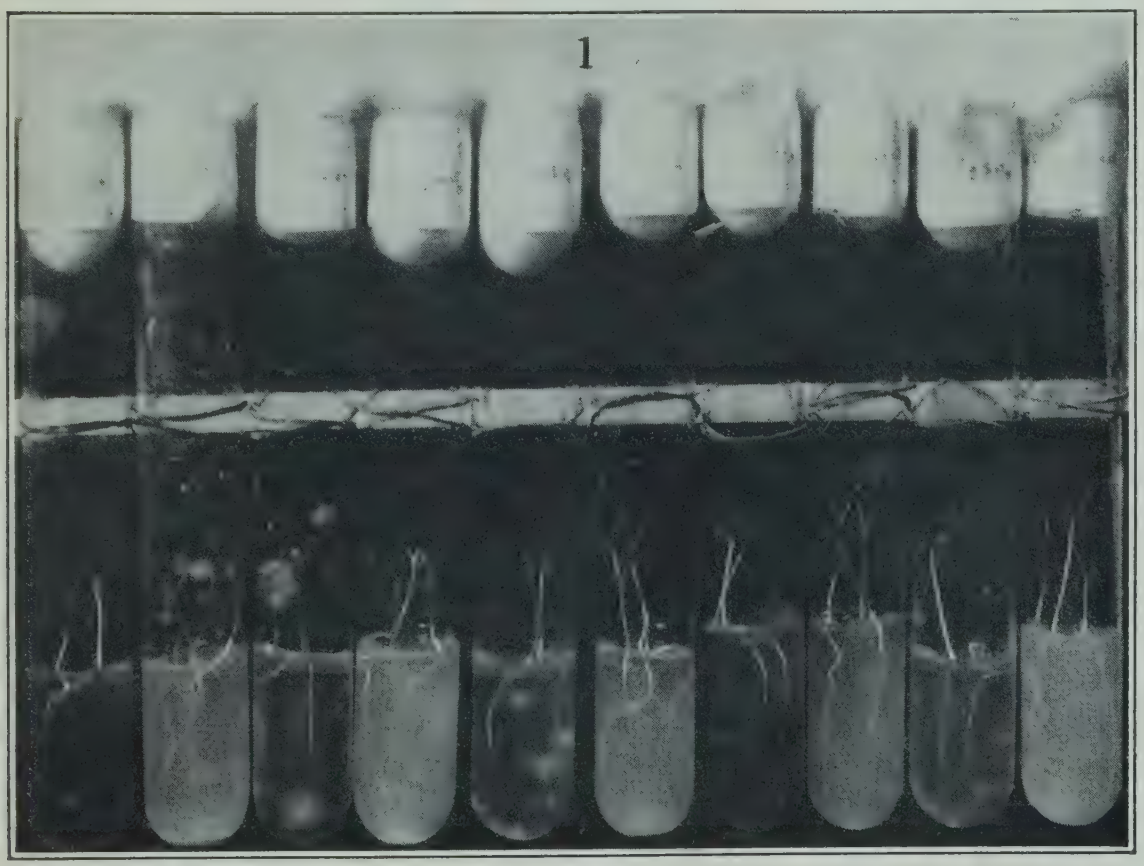
(4) The root development of red clover is increased by calcium sulphate, 0.01 per cent being apparently as efficient in producing this increase as 0.1 per cent.

(5) In small amounts calcium sulphate increases the yield of red clover and also the number of nodules. Concentration as high as 0.05 to 1 per cent, however, produces no increase in growth.

(6) The application of elemental sulphur to Miami silt-loam soil increased but slightly the yield of clover and apparently did not affect root development or nodule formation. In producing this slightly increased growth 0.01 per cent was as efficient as were higher concentrations.

(7) A review of the results of these experiments shows that calcium sulphate in soil does not produce any marked effect on the bacteria commonly found on agar plates, but does increase the growth of the legume bacteria. It also increases the yield of red clover, which is accompanied by a greater root development and a greater number of nodules.

(8) The addition of sulphur increases the ammonification, but decreases nitrification and the total number of soil organisms. It increases the yield of red clover but slightly and does not affect the root development nor the number of nodules.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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., JANUARY 24, 1916 NO. 17

A SERIOUS DISEASE IN FOREST NURSERIES CAUSED BY PERIDERMIUM FILAMENTOSUM

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In June, 1914, several seedlings of *Pinus ponderosa* Laws., with the stems severely infected with a disease caused by a species of Peridermium, were received from the Savenac nursery of the United States Forest Service, at Haugan, Mont. The seedlings were taken from the field-planting area located near the nursery. They had remained one year in the seed beds, one year in the transplant beds, and two years in the field. It seemed likely that the seedlings became infected while in the nursery, since the few yellow pines in the near vicinity of the area were free from the fungus.

On July 2, 1914, *Castilleja miniata* Dougl., growing in abundance on the nursery site, was found bearing the fungus *Cronartium coleosporioides* (D. and H.) Arthur.[1] No other species of Cronartium was found. Evidence of the æcial stage on left-over yellow-pine seedlings in the transplant beds brought the two stages in such close proximity it seemed certain that the fungus on the pine seedlings could be no other than *Peridermium filamentosum* Peck. Since the Savenac nursery has an annual output of 1,600,000 yellow-pine seedlings, it was evident that measures should be employed immediately to prevent the spread of the disease.

On May 1, 1915, all of the 2-year-old yellow-pine seedling beds were found to be infected with the fungus. The seedlings were being prepared for shipment to the planting areas in the forests, and a thorough inspection was made of all the bundled stock. All visibly infected seedlings were removed and burned. The seedlings remaining in the beds were examined, and the infected ones similarly destroyed. More than 4 per cent of the plants gave outward evidence of being attacked. Of the 10,000 seedlings inspected 432 were removed and burned. Control

[1] Meinecke, E. P. Notes on Cronartium coleosporioides Arthur and Cronartium filamentosum. *In* Phytopathology, v. 3, no. 3, p. 167–168. 1913.

methods were devised and recommended, and, as the bundling of seedlings progressed, all visibly infected trees were removed and burned. A sharp watch was kept on the beds to remove new infections as they developed.

Most of the infections were found along the north and east borders of the seedling beds. A large patch of *Castilleja miniata* was growing on the edge of a lodgepole pine (*Pinus murrayana* "Oreg. Com.") stand near the creek bank directly northeast of the infected seedling beds and not more than 200 feet distant. The records of the weather station located on the grounds show that the prevailing winds blow both northeast and southwest, which is an important factor in spore distribution between the two hosts. Thus, these winds sweep northeast over the patch of *Castilleja miniata* from the 2-year-old yellow-pine seedlings and in reversing blow from the former to the latter. In this manner the æciospores from the infected yellow pine are distributed to the castilleja plants and the sporidia borne on the castilleja leaves are transmitted to the young trees in the beds. On May 13, 1915, this fungus infection was found to be of serious importance on the yellow pine.

From fresh specimens of the blister rust brought in to the greenhouse at Missoula, Mont., two plants of *Castilleja miniata* were inoculated on May 3, 1915. These were covered with oiled-paper bags and labeled. Six control plants of the same species were potted and bagged and kept in a separate part of the greenhouse. On May 23 uredospores developed on the underside of the leaves of the two inoculated plants, while the control plants remained normal. Later the teliospores developed, sporidia being produced on May 29. Duplicate experiments were conducted at the field camp at Priest River, Idaho. Æciospores from the infected yellow-pine seedlings were sown on *Castilleja miniata* on May 14, and they gave positive results on June 11. The characteristic filamentous structure of the æcia on the pine seedlings and these transfers of the fungus to castilleja prove the fungus to be *Peridermium filamentosum* Peck.

On May 13, 1915, the native lodgepole pine surrounding the nursery was found to be infected with a trunk, a branch, and a needle form of Peridermium. The structure of the æcia of these forms indicated that the trunk and the branch forms were identical. The trunk form (known locally as the "hip canker" of the lodgepole pine) and the branch-gall form in the Rocky Mountain region have been commonly united under the name "*Peridermium harknessii* Moore."[1] Later they were transferred to *Peridermium cerebrum* Peck by Arthur and Kern.[2]

The following inoculations, made recently at Missoula, Mont., by the writers, prove that the "hip canker" and the gall-forming Peridermium of the lodgepole pine are both *Peridermium filamentosum*.

[1] Harkness, H. W. New species of California fungi. *In* Bul. Cal. Acad. Sci., v. 1, no. 1, p. 37. 1884.

[2] Arthur, J. C., and Kern, F. D. North American species of Peridermium on pine. *In* Mycologia, v. 6, no. 3, p. 133–138. 1914.

On May 17, 1915, æciospores from the "hip canker" of *Pinus contorta* from Haugan, Mont., were sown on two plants of *Castilleja miniata* under control conditions in the greenhouse at Missoula. On June 3 uredospores were present on the leaves. The teliospores appeared June 14. The two control plants remained healthy. The Cronartium was identical with that previously produced by the inoculations on *Castilleja miniata* with æciospores from the Peridermium on the 2-year-old seedlings of *Pinus ponderosa*. This demonstrates the identity of the "hip canker" Peridermium with *Peridermium filamentosum*.

The following cultural data show that the gall-forming Peridermium of the lodgepole pine is likewise identical with *Peridermium filamentosum*. On May 25, 1915, æciospores from the gall-forming Peridermium on branches of lodgepole pine were sown by the writers on three plants of *Castilleja miniata* under control conditions in the greenhouse. By June 11, 1915, uredospores had developed on the leaves, telia and sporidia being produced 10 days later. The two control plants remained healthy.

Check experiments carried on at the field camp at Priest River, Idaho, gave similar positive results. Six plants of *Castilleja miniata* were inoculated and gave positive results. All three control plants remained healthy.

Cultures, under control, made both in the greenhouse and in the field, on *Castilleja miniata* with æciospores taken from the blister rust on the lodgepole pine commonly known as *Peridermium stalactiforme* A. and K., have produced *Cronartium coleosporioides* (D. and H.) Arthur. Two plants of *Castilleja miniata* were inoculated and two control plants set aside. Both inoculated and control plants were covered with oiled-paper bags. The inoculated plants gave positive results and the controls remained healthy. This confirms the results of Meinecke [1] and the conclusions of Arthur and Kern [2] and places *Peridermium stalactiforme* without further doubt under *Peridermium filamentosum*.

The absence of oaks (*Quercus* spp.), the alternate hosts of *Peridermium harknessii* [3] and *Peridermium cerebrum*, from this region where the species of Peridermium on the lodgepole pine is so prolific, the characteristic filamentous processes in the æcia of the various forms of Peridermium appearing on the lodgepole pine, and the inoculation experiments successfully conducted on *Castilleja miniata*, all exclude the possibility of this fungus being other than *Peridermium filamentosum*.

The yellow-pine seedlings in the nursery were free from traumatic injuries. This is explained by the fact that they had remained in the same bed since germination and thus were not exposed to the injury from transplanting. All seedlings showing slight corrugations or blisterings of the lower stems gave no evidence of mechanical injury, but they

[1] Meinecke, E. P. Op. cit.

[2] Arthur, J. C., and Kern, F. D. Op. cit.

[3] Hedgcock, G. G. Notes on some western Urediniae which attack forest trees. II. *In* Phytopathology, v. 3, no. 1, p. 15–17. 1913.

developed the bright orange eruptions of the rust later. It is safe to draw the conclusion that the spore tubes which produce the infections in the seedlings penetrate the host in the absence of all surface openings due to the mechanical injuries. The period of development between the time of penetration of the host and the appearance of the æcial eruptions on the stems is about 10 to 11 months. The seedlings in question were produced from seed sown in the spring of 1913, and the spring of 1914 some of the seedlings produced the æcial eruptions. The seedlings must have been infected in the period following germination and have developed the fruiting stage in the spring of the following year. The infecting spores could have been either sporidia from the species of Cronartium on *Castilleja miniata* or possibly æciospores from the surrounding lodgepole pines infected with *Peridermium filamentosum*. Facultative autoecism in *Peridermium filamentosum* is as yet not proved, but it is suspected of being a "repeater."

During the period from May 29 to June 2, 1915, Mr. E. C. Rogers, of the Forest Service nursery at Haugan, Mont., assisted in the work of visiting and inspecting the various plantation areas near Wallace, Idaho, on the Coeur d'Alene National Forest, and those in the vicinity of Savenac nursery and Deborgia, Mont., on the Lolo National Forest. In all an area of approximately 500 acres was covered. The inspection was confined principally to the yellow-pine plots, with particular attention to the plants taken from the infected 2-year-old yellow-pine beds at Savenac nursery. Very few infections caused by species of Peridermium were recorded, some of the areas being entirely free from visible signs of the rust, although it may be present and not appear until the following year or later. In the case of the "2-year-old yellow pine, unfertilized" plot, which was planted in the spring of 1915, the few infections observed were found to be covered by the moist earth because of deep planting and thus were rendered practically incapable of spreading. Two of these infections were molded and the spores were no longer viable. The Placer Creek area near Wallace, Idaho, is a clean-burn site, the fires of 1910 having destroyed all living timber. No living pines or castilleja plants are to be found growing within a considerable distance of this area. *Castilleja miniata* and *Pinus contorta* are plentiful in the area containing 4-year-old yellow-pine seedlings located on the ridge west of the Savenac nursery. Very little visible infection was found on this plot. These facts prove the effectiveness of the inspection work in checking the spread of the disease and the necessity for culling out and burning the infected seedlings as soon as the eruptions make their appearance.

On June 1, 1915, a survey was made of the area surrounding the nursery beds for a distance of half a mile. Fifty per cent of the lodgepole-pine stand in close proximity to the beds was badly infected with *Peridermium filamentosum*. A group of 61 trees, having diameters (breast

high) of 5 inches and over and growing within 100 feet of the nursery beds, was found to be very seriously infected. Of the 61 trees, 26 had large cankers encircling the trunks varying in length from 2 to 8 feet. The branches and twigs were infected. *Peridermium montanum* was also present on the needles. *Castilleja miniata* was found growing in abundance under the trees. Lodgepole-pine seedlings in and near this area were, with rare exceptions, heavily infected with the twig and stem and the needle forms of Peridermium. Very little native yellow pine was found growing in the vicinity, most of the trees having been killed by the fires of 1910. A few veteran trees remain growing upon the ridge west of the nursery, but these show no evidence of fresh eruptions of Peridermium. These facts point to the lodgepole pine as the original distributor of infection to the yellow-pine seedling beds in the nursery.

Experiments are being conducted in an effort to control the disease. The seedlings in the nursery beds are being sprayed during the infection period. An effort is being made to eradicate the alternate host from the vicinity by mechanical or chemical means. The felling and burning of trees near by infected with Peridermium will reduce the chances of infection. The possibility of the fungus possessing facultative autoecism, the close proximity and abundance of the alternate host, and the prolific development of the same fungus upon lodgepole pine in the vicinity of the seedling beds all make *Peridermium filamentosum* a dangerous enemy to deal with in this nursery and one to be reckoned with in other forest nurseries where similar conditions exist.

SUMMARY

Peridermium filamentosum Peck has been found to cause a serious disease of yellow-pine seedlings at the Savenac nursery located at Haugan, Mont.

The various forms of Peridermium occurring on lodgepole pine at this nursery, with the exception of the foliicolous species, have been demonstrated to be *Peridermium filamentosum*, having an alternate stage on species of Castilleja.

The fact that the same species of Peridermium attacks both the lodgepole pine and the yellow pine increases the difficulty of control of this fungus.

The proximity and abundance of the alternate host (*Castilleja miniata*) of *Peridermium filamentosum* and its prolific development on lodgepole pine in the vicinity of the seedling beds tend to make this disease a dangerous one in forest nurseries.

SWEET-POTATO SCURF

By L. L. HARTER,
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INTRODUCTION

The scurf disease of the sweet potato (*Ipomoea batatas*) was first described by Halsted,[1] who published a brief account of it in 1890. To the fungus he gave the name *Monilochaetes infuscans*, a new genus and species, of which, unfortunately, he gave no technical description. For many years following his pioneer work little or no attention was given to sweet-potato diseases. This very common and interesting disease was therefore passed over until a few years ago, when the writer and others took up a study of them. For almost five years the disease has been under observation and study. It is therefore for the purpose of completing the description of the organism and recording the results of inoculation experiments and certain characteristics of the fungus heretofore unpublished that this paper is prepared.

GENERAL APPEARANCE OF THE DISEASE

Scurf is characterized by a brown discoloration of the surface of the underground parts of the sweet potato (Pl. LVII). The discolored areas may occur as spots of varying size and shape, with no definite outline, or as a uniform rusting of the entire surface. In gross appearance it reminds one somewhat of the silver scurf of the Irish potato, although it is somewhat darker. However, it does not penetrate the host to the extent that silver scurf does. The scurf of the sweet potato produces no rupture of the epidermis and is so superficial as to be easily scraped off by the finger nail.

DISTRIBUTION, PREVALENCE, AND LOSS

The writer has found the scurf very prevalent on sweet potatoes in New Jersey, Delaware, Maryland, Virginia, North Carolina, Ohio, Illinois, Iowa, and Kansas, and to a slight extent in other States. The following varieties are susceptible to scurf in varying degrees: Eclipse Sugar Yam, General Grant Vineless, Florida, Nancy Hall, Yellow Yam, Miles Yam, Red Brazilian, Dahomey, Yellow Strasburg, Pierson, Key West Yam, Vineless Yam, Southern Queen, Big Stem Jersey, Yellow Jersey, and Early Carolina. It is probable that the disease occurs on other varieties as well.

[1] Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Exp. Sta. Bul. 76, p. 25–27, fig. 17. 1890.

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Scurf is more prevalent in heavy, black soils and in soils that have been heavily manured or contain a larger amount of organic matter than in light, sandy soils.

The loss to the crop caused by the scurf is perhaps small in comparison with that caused by some of the more virulent diseases. Nevertheless, the actual financial loss throughout the country that can be attributed to this disease alone amounts to considerable. Scurfy potatoes do not command as high a price in the markets as clean ones, though if otherwise sound they are just as good for consumption. The fungus under favorable conditions, such as a relatively high humidity and temperature, continues to develop under storage conditions to a limited degree. It weakens the host, so that during periods when the storage house is rather dry the potato loses moisture and becomes shriveled and dried, rendering it unfit for sale and at the same time less resistant to the attacks of other parasites. Taubenhaus[1] claims that the fungus on the potato is easily killed by immersing for 10 minutes in a solution of mercuric chlorid (1:1,000).

ISOLATION OF THE FUNGUS

Some difficulty was experienced at first in isolating the fungus, since it proved to be a very slow grower and developed but little or not at all on some kinds of media. After some experimentation with different media it was found to make a slow growth in Irish-potato, string-bean, and oatmeal agar. By thoroughly washing the potato and disinfecting for about one minute in a solution of mercuric chlorid (1:1,000) and planting bits of the tissue in plates of oatmeal agar by means of sterile instruments a pure culture could generally be secured. In a week or 10 days transfers were made to media in test tubes, usually cooked rice in water or sterile, moistened corn meal. At the end of three or four weeks on these media a matted growth of dark-brown hyphæ developed. Hyaline spores are produced in abundance on long, stout conidiophores in tubes of cooked rice.

INOCULATION EXPERIMENTS

Inoculation experiments were begun on October 13, 1914, and performed as follows: Sound potatoes were thoroughly washed in water and placed in moist chambers with moistened filter paper in the bottom. They were then sprayed with a suspension of spores and bits of broken hyphæ of the scurf fungus in sterile water and exposed to laboratory room conditions. Water was added from time to time, as necessity required, to maintain the humidity of the moist chamber. At the end of two weeks small centers of infection appeared indiscriminately over the surface of the potatoes. These centers gradually enlarged, either by the merging of two or more spots or by the enlargement from a single center. There is undoubtedly considerable enlarging of the spots in moist chambers from

[1] Taubenhaus, J. J. Soil stain and pox, two little known diseases of the sweet potato. (Abstract.) *In* Phytopathology, v. 4, no. 6, p. 405. 1914.

centers of infection, in view of the fact that conidiophores often 200μ in length stand erect or at an angle on the surface of the potato and drop their spores, starting new infections outside the point of original growth. The spots, however, so far as the writer has been able to determine, do not enlarge by the branching and creeping of the hyphæ over the surface. Repeated inoculation experiments gave similar results. The checks remained free from the disease.

DESCRIPTION OF THE FUNGUS

The young vegetative growth of *Monilochaetes infuscans* is hyaline and septate. At the end of a few days, however, with the exception of the terminal cell of the conidiophore, the hyphæ turn densely brown. On the host little or no branching of the vegetative growth takes place. Although Halsted figured a branching of the hyphæ which was hyaline in color within the tissues of the host, the writer, after long and detailed examination of paraffine sections and sections prepared in other ways, has not been able to find a sure example. The sporophores, for such they appear to be, arise from the surface of the host and are attached to it by an enlarged end cell slightly buried in the cuticle (Pl. LVIII, *E*, *C*, *D*). Occasionally a second (Pl. LVIII, *I*) or third (Pl. LVIII, *J*) enlargement or bulblike growth is found deeper in the host or parallel with the surface (Pl. LVIII, *G*). From some of these secondary enlargements a conidiophore may be developed (Pl. LVIII, *F*, *H*). Plate LVIII, *E*, *C*, shows conidiophores bearing conidia produced on the host. The brown septate conidiophores vary in length from 40 to 175μ and bear at the end a single-celled spore, which on the host is slightly brown or hyaline. The conidia are 12 to 20μ in length by 4 to 7μ in thickness.

This fungus, as might be expected, behaves differently when grown artificially. Growth has been carefully observed on a few of the common media—namely, Irish-potato agar, beef agar, rice agar, oatmeal agar, string-bean agar, Irish-potato cylinders, sweet-potato stems, and stems of *Melilotus alba*. At the end of 24 days a very slight growth appeared on string-bean agar, rice agar, and oatmeal agar at a temperature varying from 6° to 7° C. Conidia were very sparingly produced. At room temperature (23° to 26°) growth was visible on all media in 4 days, except on rice agar and the stems of sweet potatoes and *Melilotus alba*. In 13 days a small growth appeared on rice agar, but on stems of sweet potatoes and sweet clover no growth was detected at the end of 4 weeks. There is very little difference in the gross appearance of the growth on any of the media used. Enlargement from a single center is very slow, attaining a diameter of about 2 to 5 mm. in 14 days. The fungus piles up in an almost black feltlike mass 2 to 3 mm. in height, with an entire margin. It penetrates the medium but little. The vegetative hyphæ in mass are almost charcoal-black, although in gross appearance there is some variation on different culture media. On Irish-

potato cylinders and Irish-potato agar the growth has a darker appearance than on oatmeal agar, beef agar, and string-bean agar, owing to the fact that the numerous erect conidiophores bearing hyaline spores are produced in greater abundance on the three latter media and give a grayish appearance to the upper surface. If the conidiophores and spores be scraped away, the mass is black beneath. Growth appeared only on oatmeal agar at temperatures varying from 30° to 32° in 14 days. From these results it appears that temperatures as low as 6° to 7° and as high as 30° to 32° prohibit the normal growth of the fungus.

The vegetative growth on artificial cultures is hyaline at first and later brown (Pl. LVIII, *L*), with the exception of the end cell of the conidiophore, which at its outer extremity is hyaline to slightly brown (Pl. LVIII, *A*, *B*, *L*). The conidiophores are branched, septate (Pl. LVIII, *A*, *L*), and vary in length from 30 to 225μ. The conidia are continuous, granular, and hyaline to slightly brown with age (Pl. LVIII, *M*). As soon as one conidium is mature, it separates easily from the conidiophore and another begins growth by a swelling of the end cell of the conidiophore, to be dropped in turn when mature. This process is repeated as long as the environment of the host will permit. It should be noted in this connection also that this fungus can be reproduced by hyphæ as well as from the spores. It is likely also that vegetative reproduction accounts for a larger part of the infections under natural conditions. In fact, certain vegetative parts might be confused with or mistaken for conidia. Although conidia are not produced in abundance on the host, they frequently develop normally on diseased potatoes kept for some days in a moist chamber.

The conidia under laboratory conditions germinate slowly in rice or sweet-potato decoction. One or two growths (Pl. LVIII, *K*) are thrown out usually at the end of the conidia, which attain in 24 hours a length about equal to that of the spore. The branching of the hyphæ begins the second day (Pl. LVIII, *N*), and the production of the brown pigment in about three days.

TAXONOMY OF THE FUNGUS

Halstead attributed the scurf to a new genus and species, *Monilochaetes infuscans*, but he gave no technical description of it that the writer has been able to find. The fungus belongs to the Dematiaceae of the Hyphomycetes. However, the writer has been unable, after considerable study of the fungus, to fit it into any of the genera so far described. It is, however, desirable, in view of the fact that it is a rather common and conspicuous fungus, that it have a description by which it may be recognized. The fungus has been known as *Monilochaetes infuscans* and as the cause of the sweet-potato scurf for 25 years. Taubenhaus and Manns [1] in a recent publication likewise refer to *Monilochaetes infuscans*

[1] Taubenhaus, J. J., and Manns, T. F. The diseases of the sweet potato and their control. Del. Agr. Exp. Sta. Bul. 109, p. 11. 1915.

as the cause of the disease. In view of these facts, it is believed preferable to give it a description and permit it to maintain generic rank rather than to place it in a genus where it does not naturally belong.[1]

MONILOCHAETES

Hyphæ dark, erect, rigid, septate, not in definite fascicles; conidia distinctly different from the sporophores and hyphæ, hyaline, slightly brown with age, continuous, not in chains, acrogenous.

Monilochaetes infuscans

On the host definite vegetative hyphæ are lacking; sporophores septate, erect, unbranched, dark, and attached to the host singly or by twos, by a bulblike enlargement 40 to 175μ long, 4 to 6μ wide, bearing rarely a hyaline one-celled oblong spore. In cooked rice the hyphæ are much branched, septate, brown; sporophores brown except at terminal cell, which is frequently hyaline to slightly brown, septate, branched, stout, 30 to 225 by 4 to 6μ; conidia abundant, one-celled, hyaline, ovoid to oblong, 12 to 20 by 4 to 7μ, solitary, terminal.

Parasitic on the underground parts of *Ipomoea batatas*. Type specimens deposited in the pathological collection of the herbarium of the United States Department of Agriculture, Washington, D. C.

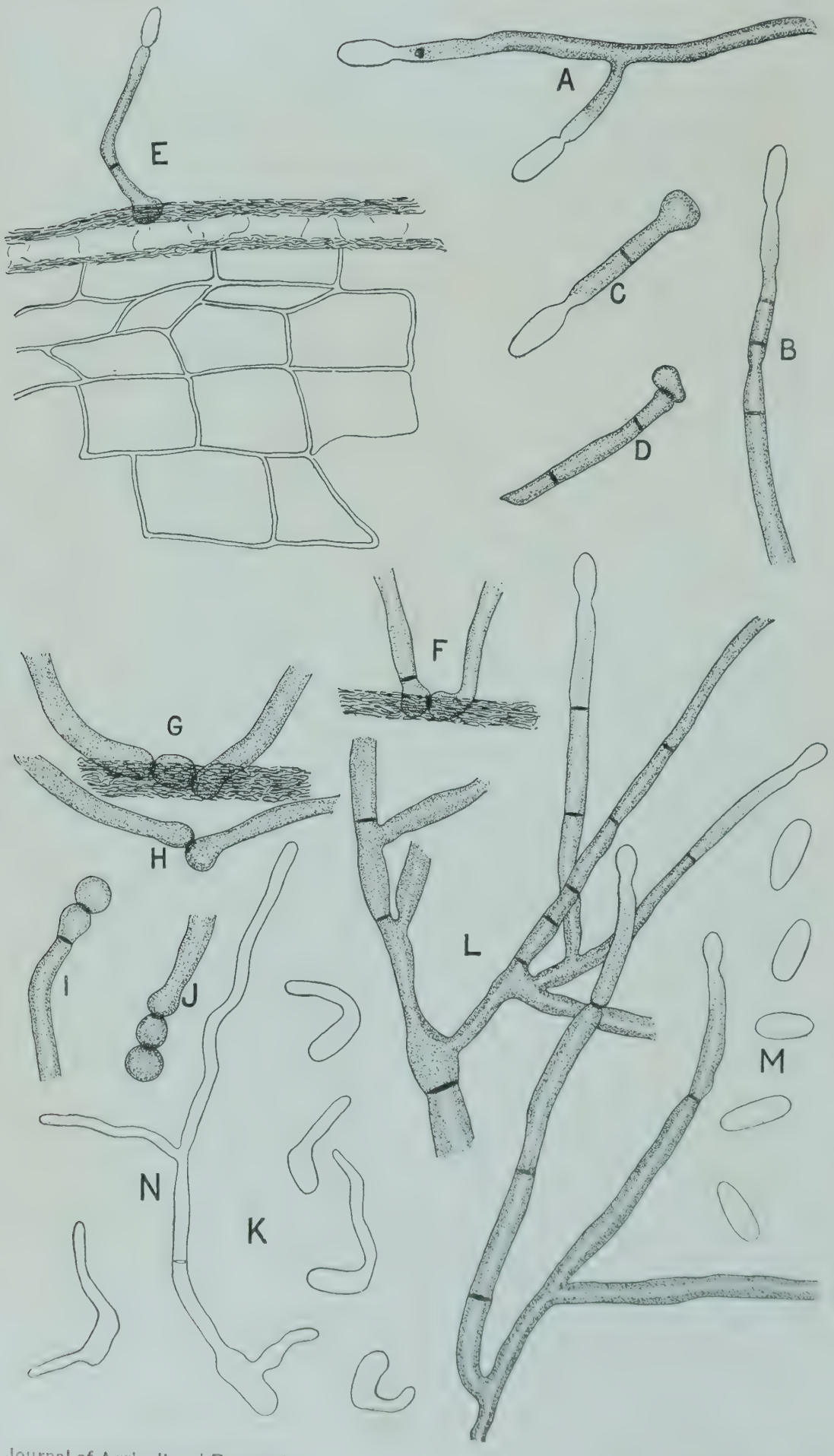
SUMMARY

The scurf disease of the sweet potato was first recognized in 1890 by Halsted, who named the fungus "*Monilochaetes infuscans*," a new genus and species. He failed, however, to describe either the genus or species. The scurf has been found prevalent in nine States and sparingly in others, and on 16 varieties of sweet potatoes. The organism has been shown by inoculation experiments to be the true cause of the disease. A detailed discussion of the morphology of the organism is taken up, also its growth on different culture media at different temperatures. It was found that the organism on the host consisted merely of sporophores and conidia. In culture, however, well-defined branched mycelia and spores developed.

[1] The writer is indebted to Dr. C. L. Shear and Mrs. Flora W. Patterson, of the Bureau of Plant Industry, for having examined specimens of this fungus.

PLATE LVII

A sweet potato showing the discoloration produced by *Monilochaetes infuscans*.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BANANA AS A HOST FRUIT OF THE MEDITERRANEAN FRUIT FLY

By E. A. Back, *Entomological Assistant*, and C. E. Pemberton, *Scientific Assistant*, *Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

INTRODUCTION

The banana export trade of the Hawaiian Islands amounted to 256,319 bunches of Chinese bananas (*Musa cavendishii*) during the year ending June 30, 1915. Although 25,448 bunches were shipped during June, 1915, the monthly average for the year was 19,621. With such a trade with the California coast established, it became imperative to determine to what extent bananas are infested by the Mediterranean fruit fly (*Ceratitis capitata* Wied.), in order that data might be placed on file for the guidance of the Federal Horticultural Board in forming its quarantine regulations for the protection of mainland fruit interests. While it has been proved that bananas may serve as host fruits of this fruit fly when ripe, all data happily corroborate the general belief among shippers and growers, as well as among entomologists familiar with the situation, that Chinese bananas and Jamaica or Bluefield bananas (*Musa* spp.), when cut and shipped under commercial conditions, are immune to attack and offer no danger as carriers of this pest if properly inspected and certified as provided for by the regulations of the Federal Horticultural Board (8).[1] These regulations, it may be stated, provide for inspection in the packing sheds for the presence of prematurely ripe, bruised, cracked, and decayed fruits; require the use of safe packing material; and prohibit the shipment of bananas from plantations the surroundings of which have not been favorably passed upon from a fruit-fly standpoint by a representative of the Board.

EVIDENCE FROM TRAPS AS TO THE PRESENCE OF ADULT FRUIT FLIES IN BANANA PLANTATIONS

The establishment of a series of traps among banana plants has shown that adult fruit flies are everywhere present in banana plantations in Hawaii. Traps were placed in the Moanalua, Moiliili, Waikiki, Mokuleia, Kawaihapai, and Puuiki plantations. As many as 793 adult flies were taken in one trap suspended from a bunch of bananas in a field at Moanalua between July 28 and August 7, 1913. Traps hung in the much larger and exceptionally well isolated banana fields of Puuiki, Kawaihapai, and Mokuleia in the Waialua district of Oahu showed a

[1] Numbers in parentheses refer to "Literature cited," p. 803.

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far smaller number of adults, yet a sufficient number to infest bananas were they readily subject to infestation. In this district 57 traps caught no flies between August 9 and 21, 1913, while the average for the same period for 119 traps in which flies were caught amounted to 7.5 adults. Flies were taken in all traps hung at Moanalua, Waikiki, and Moiliili, although some of the traps were hung in the center of the largest blocks of trees. At Moanalua as few as 22 and as many as 3,334 adult flies were taken from individual traps between July 15 and August 29, 1913, while at Waikiki and Moiliili as few as 1 and as many as 402 adults were taken between June 17 and July 8, 1913. Thirty-six was the largest number of flies taken from any trap at Waialua between August 9 and 21, 1913. Although only males were caught in the traps, adults caught in the hand net showed the sexes to be present in the usual proportion among the banana plants. These data determine at once the fact that the general immunity of bananas is not due to any lack among banana plants of adult fruit flies capable of ovipositing.

ABSENCE OF INFESTATION AMONG RIPE AND GREEN BANANAS, AS EVIDENCED BY FIELD INSPECTIONS AND LABORATORY REARINGS

During the period of somewhat over three years that the Federal Government has had supervision of the inspection of export bananas in the Hawaiian Islands (from August, 1912, to the present time) the writers have seen no case of infestation among ripe or green bananas grown under normal field conditions, and neither have the banana inspectors. Frequently individual fruits on a bunch of bananas will ripen in advance of the other fruits. When the bunches are cut, these prematurely ripe fruits, which often in addition have the peel split so as to expose the pulp, are removed before shipment and discarded at the packing sheds. If any bananas are subject to infestation, it would seem that these fruits are most likely to be; yet 1,044 prematurely ripened fruits brought to the laboratory during 1913 and 1914 and placed in rearing jars yielded no adult flies, although they came from fields known to harbor adult flies. During August, 1914, when large numbers of flies were maturing from peaches in a garden in Manoa Valley, fully ripe Chinese bananas, and a variety known to the Hawaiians as the apple-banana (*Musa* sp.), growing in the midst of other species of infested fruits, showed no infestation. Thirty-nine fully ripe apple-bananas grown near the insectary from which flies were continually emerging showed no infestation. An examination of 27,000 fruits of the Chinese banana ready for shipment at several banana fields at Moanalua during early July, 1913, when records showed the adult flies to be very abundant, failed to reveal a single distinct egg puncture. Even suspicious abrasions were investigated and found not to extend through the skin nor to contain fruit-fly eggs. An examination of 3,500 similar fruits at Kalauao during July, 1913, also gave negative results. No fruit flies have been reared from about 1,000 green Chinese bananas

discarded at time of shipment at the packing sheds because of split peelings or black decayed ends. Fifty fruits of the Hawaiian variety, known as the "ice-cream" banana (*Musa* sp.), cut from the tree as they were turning color, showed no infestation, though growing in the midst of other species of infested fruits. No infestation was found among 500 overripe fruits of the Manila Hemp banana (*Musa textilis*) growing near the corner of King and Punabon Streets, Honolulu, nor among 60 fruits of the Borabora banana (*Musa fehi*), known to the Hawaiians as the Polapola banana, in a ripe though not soft condition, growing in a mountainous ravine at the head of Manoa Valley, Oahu.

There are no records of infestation of the Chinese and Bluefield bananas grown under commercial conditions in the Hawaiian Islands, or developing and ripening in city lots.

INFESTATION OF POPOULU AND MOA VARIETIES

The only case of infestation among bananas growing in the field was brought to the attention of Mr. David Haughs, of the Territorial Board of Agriculture and Forestry, on October 17, 1913. The infested fruits were of the Popoulu and Moa varieties (2) of the Popoulu group (*Musa* spp.) of cooking bananas. These are short, thick bananas, with comparatively thin skins. They are never eaten raw and, unlike the Chinese or Bluefield bananas, are rarely, if ever, shipped from the islands. They are very scarce and are strikingly distinct both from the ordinary cooking banana and from the banana of commerce.

Of the 11 fruits on the bunch of Popoulu bananas when the examination was made 7 were still green, though on the point of turning yellow, and 4 had turned yellow. There were in the peel no splits nor mechanical injuries and there was every evidence that the punctures found in three of the four ripe fruits had been made while the bunch was still on the tree. Mr. J. C. Bridwell, of the Hawaiian Board of Agriculture, had charge of the rearing, but kept no definite record of the number of adult flies reared from infested fruits. That larvæ matured and emerged from one fruit at least is evidenced by the numerous emergence holes in the peel (Pl. LIX, fig. 1).

The Moa variety was growing in the same garden with the Popoulu banana. The fruits of this variety are much larger and the peel thicker. Of 9 fruits taken from the single bunch found, 5 were perfect, but the peel of the 4 other fruits was so cracked that the pulp was well exposed; all were green in color but mature and about to turn yellow. Mr. Bridwell's notes, which have been placed at the writers' disposal through the courtesy of the Territorial authorities, state that of 12 distinct attempts at oviposition made in the peel of the 4 sound fruits, only one puncture was sufficiently deep to contain eggs, but no eggs were deposited. Only one of the 4 cracked fruits developed larvæ, and the eggs from which

these hatched were laid directly into the pulp along the crack in the peel. Of the punctures found in the peel of the cracked fruits, only one contained eggs, and these were dead and shriveled. Mr. Bridwell kept no definite record of the number of adult flies reared, but it was large. He estimates that from the Popoulu and the Moa fruits he reared about 350 adults. The thoroughness with which the larvæ destroyed the pulp of the Moa banana is shown in Plate LIX, figure 2.

Special attention should be called to the fact that infestation of the pulp in these two varieties occurred only in the fully ripe and yellow fruits of the Popoulu variety, which has a very thin skin, and in the fruits of the Moa variety, the peel of which was cracked, thus removing from the exposed pulp beneath the natural barrier to infestation referred to below. The ordinary cooking bananas, such as are in general use in the islands, are quite unlike the Popoulu and Moa varieties in shape.

EXPERIMENTS TO FORCE INFESTATION

While infestation of Hawaiian bananas has never been known to occur among fruits grown and harvested in accordance with trade requirements and prepared for shipment in accordance with the regulations of the Federal Horticultural Board, experiments have been carried on under more or less artificial and abnormal conditions for the purpose of determining whether the general immunity of commercially grown bananas in Hawaii is due to the presence of other host fruits for which the fruit fly has a greater preference or to some characteristic which renders them actually immune. Such experiments have been completed both in the field and in the laboratory.

EXPERIMENTS IN THE FIELD

As the writers have found that in the field they can bring about an infestation of ripe bananas, or in the laboratory of green but well-grown bananas that have been cut from the tree so long that the protecting sap has ceased to flow to any extent, their field experiments have been confined mainly to forcing, if possible, an infestation of bananas still attached to the tree yet sufficiently mature for the export trade.

During March, 1913, a rearing cage, 9 by 15 by 24 feet, was built over 20 Chinese banana trees bearing 14 bunches of bananas. Into this cheesecloth-covered cage (Pl. LXII, fig. 1, 2) were introduced from time to time a total of over 3,000 Mediterranean fruit flies. The foliage within the cage was sprayed every few days with a solution of pineapple juice and water, as there was nothing else upon which the fruit flies could feed. As the fruits on the various bunches ripened, they were cut and placed in rearing jars in the insectary. The 14 bunches represented approximately 1,000 fruits, which ripened over a period extending from the middle of March to June 28. No adult flies developed from any of this fruit.

In order more closely to confine gravid females with bananas ripe enough for shipment, a fine wire cylinder, 20 inches in diameter and

30 inches long, closed at each end by cheesecloth, was placed over the entire bunch. From 200 to 500 fruit flies were introduced through the lower opening and allowed to remain with the fruit from 24 to 48 hours. The cage was then removed, the bunch cut, and the individual fruits examined for evidences of oviposition. Out of a total of 1,449 fruits thus carefully examined, 1,363 showed no evidence of attempted oviposition, while 86 bore puncture marks. In the peel of these 86 fruits the females had made 169 breaks in attempts to oviposit. Only two punctures were sufficiently deep to permit oviposition, and of these only one contained a single egg. This egg was deposited between August 21 and August 23, 1913, and by August 27, when the examination was made, fully two days after the egg should have hatched under normal conditions, it was found dead and blackened. None of the other attempts at oviposition extended for more than one thirty-second of an inch below the surface, while nearly all were mere abrasions. In all cases, however, each break in the skin was surrounded and quite well sealed by dried, sticky exudations. In a few instances the sap flowed from 1 to 2 inches down the side of the fruit from the puncture.

Before bunches of bananas are cut in the field they are stamped by the official marker of the shipper. Ten bunches stamped on June 21 were allowed to remain growing to determine whether the development that takes place during a 10-day period after the fruit is sufficiently mature for shipment lessens the general immunity it enjoys if cut when marked. It should be stated here that unless bananas are cut for shipment on the steamer for which they are marked they become too mature or, to use trade terms, too "full" or "fat," to stand without decay the 9- to 14-days' interval before they are exposed for sale in the San Francisco market. Only 9 fruits out of 505 on 4 of these 10 bunches caged with fruit flies between June 21 and June 23 bore evidences of attack, there being such evidence in 14 places. All punctures were empty, except one containing 5 eggs. These eggs had been laid in a crack caused by the decay of the blossom end of the fruit. While these eggs hatched, the larvæ immediately died. Out of 238 fruits on 2 bunches caged with fruit flies between June 23 and 26, 42 showed 159 breaks in the peel made by flies. Of these only 3 contained eggs—3, 4, and 6, respectively. An examination of these eggs on July 7 showed that while they had hatched, the larvæ were not able to mature and had died in the punctures. There were 126 attempts at oviposition in 46 out of 202 fruits on 2 bunches caged with fruit flies between June 26 and June 28; of these punctures only 2 contained eggs—1 and 3, respectively. While 3 of these eggs hatched, the larvæ died without entering the pulp. No eggs were found in 26 punctures in the peel of 15 out of 200 fruits on the last 2 bunches of those marked "June 21," and caged with fruit flies between June 28 and June 30. Plate LXI, figure 2, is reproduced from a photograph of the blossom end of a Chinese banana taken 16 days after it was

marked for shipment. The 18 punctures found on this fruit were made between June 28 and 30, or 7 to 9 days after the fruit was marked for shipment. All of these punctures were empty, and only 2 were sufficiently deep to contain eggs. The dried exudations have been removed.

Having failed to force Mediterranean fruit flies to oviposit successfully in the field in bananas sufficiently mature for the export trade, freshly laid eggs were removed from apples and placed in incisions made in the peel of bananas marked for shipment but still attached to the tree. Small cuts varying from one-fourth to one-half inch in length, extending with the grain of the peel but not quite reaching the pulp, were made. From these cuts the sap flowed so freely that it was difficult to insert eggs quickly enough to prevent them from being washed away. A total of 470 eggs inserted were sealed within the incisions with gummed labels and a thin layer of paraffin. Upon the examination of 270 eggs 2 days later, it was found that 60 eggs had hatched and that the newly hatched larvæ were alive and active within the incisions. Later examinations showed that all larvæ died without entering the pulp, even where the peel had split and exposed the latter. An examination of the 200 other eggs 9 days after they were placed within the incisions showed that 135 had hatched, but all the larvæ had died without infesting the pulp. The 275 of the 470 eggs that failed to hatch turned black. Of 65 eggs of the same lot held as a check, 57 hatched.

EXPERIMENTS IN THE LABORATORY

All experiments carried on in the laboratory necessarily were with fruits cut from the tree. The results were therefore obtained under conditions less normal than those obtained in the field. No experiments can be said to be carried on under field conditions unless the fruit is still growing, for as soon as it is cut its protecting sap begins to disappear.

One bunch of 55 fruits which had been cut for shipment for 24 hours was confined for 48 hours with about 500 fruit flies. An examination of the individual fruits after the bunch was removed from the cage showed 22 with a total of 28 punctures. These punctures were not opened, but the fruits were placed in jars. No adult fruit flies developed.

One bunch of 93 fruits, which had been cut for shipment for about 6 hours, was confined for 24 hours with about 300 fruit flies. On removal from the cage it was found that only 15 fruits were free from attempts at oviposition. In the remaining 78 fruits there were 342 punctures. Eggs were laid in only 7 of these 342 punctures. All eggs, or newly-hatched larvæ, died in 5 of the 7 punctures and only 3 adult flies succeeded in developing, in but one of the two fruits the pulp of which was found infested 5 days after the fruit was removed from the cage. The fruits on this bunch were almost too mature for shipment.

Twenty fruits from a bunch cut four days previously for shipment were confined in a jar containing about 400 fruit flies. Five fruits were

removed after 24 hours; 15 fruits after 72 hours. At the end of the 72 hours, or 7 days after the fruits were cut, they were beginning to turn color. In the peel of the 5 fruits first removed 58 punctures were made; yet only 1, 3, 2, and 1 fruit flies, respectively, were reared from 4 of the fruits. In the peel of the 15 fruits removed at the end of 72 hours there were 148 punctures, of which 28 contained eggs. Two days after the fruit was removed from the jars, the 28 punctures were found to contain 59 hatched eggs and 27 dead eggs. While punctures were found to be entirely empty in only 2 of the 15 fruits, adult fruit flies failed to mature in 7. There issued from the remaining 8 fruits an average of 2.2 flies, 8 being the largest number to emerge from a single fruit. Two fruits, found to contain 18 and 19 eggs, respectively, failed to produce adults.

Three fruits of the wild Borabora banana, which had been cut from the tree for two days and were still hard and yielding small quantities of sap when cut from the bunches, were placed with about 200 fruit flies for 24 hours. After removal from the cage, one fruit contained 56 eggs in its peel. The two other fruits were placed in rearing jars and produced 104 and 187 adult fruit flies, respectively. The pulp of the Borabora banana is very firm and does not decay as rapidly as does that of the Chinese or Bluefield banana.

Only 35 adults matured from 880 eggs taken from apples and placed in the peel of 44 bananas that had been cut for shipment for 24 hours. Of the 44 fruits only 31 produced adult fruit flies. Out of 107 newly hatched larvæ from apples, placed in the pulp of ripe bananas, but 33 succeeded in reaching the adult stage. Out of 137 newly hatched larvæ placed in the pulp of green bananas ready for shipment, but 40 completed the life cycle. Of these 137 larvæ 15, 52, 60, 26, and 10 were placed in bananas that had been cut from the tree 1, 2, 3, 4, and 9 days, respectively; the adults reared in the same order numbered 3, 12, 13, 5, and 7

CAUSES OF IMMUNITY OF GREEN BANANAS TO FRUIT-FLY ATTACK

While it is difficult to understand why Mediterreanean fruit flies have not been reared from ripe and split fruits collected on the plantations, it is not so difficult to find reasons for the immunity of fruits until they are about to turn yellow. Chemical analysis of the banana during its development, made by Mr. A. R. Thompson, of the Hawaii Agricultural Experiment Station, have shown that there exists much tannin in the peel and about the sections of which the banana fruit is composed. This tannic acid is very abundant in the green fruit, but decreases greatly in amount as the fruit becomes edible. During development, even up to the time when bananas are cut for shipment, which usually is about 12 to 16 days before they would become ripe enough to eat if kept under Hawaiian weather conditions, the peel of the fruit is so surcharged with sap laden with tannic acid that the slightest scratch of the peel produces

a flow of this staining fluid. Data on file show that practically all punctures made by female fruit flies in host fruits, the epidermis of which does not emit fluid detrimental to the pest from one or several standpoints, contain eggs, but no punctures or eggs have ever been found by the writers in the peel of bananas growing under normal field conditions and suitable for the export trade. This is true in spite of the fact that many thousand fruits have been examined.

One of the most severe tests to which any fruit can be subjected to determine whether it can support the fruit fly is to confine it closely with several hundred fruit flies of both sexes. Yet even under this extreme and unnatural condition only 1 egg was laid in 1,449 bananas exposed while still attached to the tree, and that was killed, presumably by the tannic acid in the peel. While 22 eggs were deposited in 1,145 more mature fruits, also attached to the tree, some of which were too mature for export trade, these eggs, or the larvæ hatching from them, died within the peel. When one realizes that many thousand eggs have been secured by the writers under like conditions in preferred hosts, it is clear that adult fruit flies find it extremely difficult to oviposit in fruits on the tree even under forced conditions, both when the fruit is sufficiently mature for shipment and for a period of at least nine days thereafter. At the end of this period it is considered too mature to stand transportation to the mainland. And inasmuch as shippers are paid by the bunch for their fruit, the banana markers in Hawaii are likely to mark bananas for cutting that are slightly greener than necessary in order to safeguard against unforeseen delays in shipment and crowded conditions on board the steamer which hasten the ripening process.

The difficulty experienced by the female Mediterranean fruit flies in ovipositing in green though mature fruit still attached to the tree is undoubtedly a mechanical one. She no sooner ruptures the epidermis in her attempt to form a cavity within which to deposit her eggs than she is literally forced away from her position by the exuding sap. It is possible that repeated attempts at oviposition, which are known to occur in other host fruits under natural conditions, may account for the 7 instances out of the 494 under forced or abnormal conditions when females were successful in depositing eggs. That the immunity enjoyed by Chinese and Bluefield bananas up to the time they are ready for shipment and for a period of at least nine days thereafter is due to the copious supply of sap is still further emphasized by the ease with which they become infested under similar forced conditions, or outdoor conditions, when the fruit has been cut for a short time. Fruit cut from the tree or from the bunch bleeds at the point where severed. The pressure of sap within is at once reduced and the amount of sap that exudes from cuts in the peel decreases until but little exudes after the fruit has been cut for several days. The data giving the results of close confinement of flies with bananas after they have been cut for shipment show that while the females have diffi-

culty in ovipositing as abundantly as they would in preferred hosts, such as the apple and peach, yet they find little difficulty in depositing a sufficient number of eggs to infest slightly a few of the fruits.

Inasmuch as not a single egg or newly hatched larva, as recorded in the data, was able to live in the tannin-laden peel of green though mature bananas still attached to the tree, while adults were frequently able to reach maturity in fruits severed from the tree, from which much of the sap had been drained or altered by chemical changes that proceed with the ripening process, it is evident that the sap is the chief cause of the immunity of bananas to the attack of *Ceratitis capitata*.

There is no danger of infestation during the interval between the time bananas are cut in the field and the time they are wrapped for shipment in the packing sheds.

It has been noted that oviposition has taken place under forced conditions within from 6 to 24 hours after the fruits have been cut from the tree, but that eggs deposited under such conditions have either died or the larvæ hatching from these have died without reaching the pulp. This leads to the question whether there is not danger of bananas becoming infested between the time when they are cut and the time when they are wrapped. The writers have never seen adult flies resting on bananas cut and stacked in the packing sheds, although they have personally seen many thousands of bunches ready for inspection during a 3-year period. Trade requirements demand that fruits be cut as late before the date of steamer sailing as possible. It therefore happens that bunches of bananas are inspected and wrapped within from 2 to 24 hours after they are cut, and this prompt wrapping removes all danger of infestation (Pl. LX, fig. 1, 2). From the fact that no infestation of growing bananas in condition for shipment has been known to occur in Hawaii, and that such infestations in cut fruits also suitable for shipment that are recorded have been obtained under forced conditions, whereas they have been found lacking under normal conditions, the writers believe that there is no possibility of infestation taking place between the time of cutting and that of wrapping.

OBSERVATIONS AND EXPERIMENTS OF OTHER ENTOMOLOGISTS

Kirk, of New Zealand, lists (4) the banana among fruits from Australia, condemned in New Zealand, in which the maggots of the fruit fly[1] had

[1] From the arrangement of the text of Kirk's bulletin (4), the Mediterranean fruit fly (*Ceratitis capitata*) is definitely listed as a banana pest. The bulletin is, however, a compilation taken for the most part verbatim from various articles on fruit flies appearing in the Reports of the Agricultural Department of New Zealand, or from circulars issued by the department. A person unfamiliar with the Australian situation is at a loss to know to which of several fruit-fly pests reference is made in the reports of fruits found infested by maggots at the ports of entry. Thus, in the Thirteenth Volume of the Agricultural Reports, 1905, where the list including the banana among those fruits found infested was originally published, no reference is made to either the Queensland or the Mediterranean fruit fly; it is merely stated that the fruits listed were burned because found infested with the "dreaded maggot." In the report for 1906 it is definitely stated that only the Queensland fruit fly (*Dacus tryoni*) was reared that year from a list of fruits including the banana. The biologist of Western Australia in his report (1) for the year 1898 stated that the Queensland fruit fly had been brought to Western Australia in bananas.

been found. French, of Victoria, Australia, states (3) that adults of this pest were reared from bananas (*Musa* sp.) exported from Queensland, Australia, and that on many occasions he has proved eggs to have been deposited in green bananas before shipment from Queensland to Melbourne. Both Kirk and French are aware that the Queensland fruit fly (*Dacus tryoni*) is a pest of bananas grown in Queensland and that confusion between the two fruit flies might occur if observations were made by untrained inspectors.

The only actual data, aside from those presented in this paper, giving the results of experimental work to determine the status of the banana as a host fruit of the Mediterranean fruit fly have been presented by Severin and Hartung (5, 6). This work was done in Honolulu and the results are of such value that they should be consulted by those interested. Their experiments, however, were carried on with fruits detached from the tree, and when green fruits were used no statement regarding the degree of greenness was made. In view of the fact that they reared specimens of the fruit fly from only two fruits out of "hundreds of bunches of bananas" examined on trees cut down in Honolulu during a campaign against mosquitoes, the writers seriously question the statement made by Severin in a later publication (7) that the "fruit fly was also bred from a half-ripe banana under field conditions." The fact that Severin reared numerous specimens of the decay flies, *Acritochaeta pulvinata, Euxesta annonae* Fab., and *Notogramma stigma* Fab., besides a number of species of Drosophilidae, is ample evidence that the trees from which the two fruits were taken had been cut sufficiently long for decay to have started in many fruits, had he not stated that one of the two fruits from which he reared adult flies was in a bruised and decaying condition and that its pulp had already turned yellow beneath the decayed area. It is general knowledge in Honolulu that such quantities of bearing banana trees were cut down during the campaign mentioned that the city garbage department was completely demoralized and that the trees with their fruit attached were stacked along the streets in certain parts of the city for over a week, thus giving fruit flies an opportunity to oviposit under, not growing or field, but abnormal conditions.

CONCLUSIONS

Since the Mediterranean fruit fly (*Ceratitis capitata* Wied.) has not been found infesting the Chinese banana (*Musa cavendishii*) or the Bluefield banana (*Musa* sp.) during the three years that the Federal Government has had charge of the inspection of export bananas in the Hawaiian Islands, it is evident that some reason exists for this practical immunity. This is the more apparent since adult flies of both sexes have been found present in all parts of banana plantations, and surrounding fruits known to be hosts have been heavily infested.

This immunity is shown to be due to the fact that neither the egg nor the newly hatched larva of the fruit fly can survive in the tannin-laden peel of green though mature fruit. In fact, the copious and sudden flow of sap from egg punctures made by fruit flies in unripe bananas renders the successful deposition of eggs in such fruits difficult and rare.

The fact that not 1 of 1,044 fruits of the Chinese banana ripening singly and prematurely among bunches growing in the field, and upon which, as in the case of other host fruits, one might expect gravid females to concentrate their attention for the purpose of oviposition, has been found to be infested leads to the conclusion that even ripe bananas are not desired as host fruits by adult fruit flies under Hawaiian conditions. On the other hand, the rearing of flies from the ripe and yellow fruits of the thin-skinned Popoulu variety, as well as from ripe fruits of other varieties under forced and unnatural conditions, leads to the equally acknowledged fact that ripe bananas in the field may serve as hosts and should therefore be properly guarded against in all quarantine work.

From the facts stated the writers believe that bunches of any variety of banana now growing in the Hawaiian Islands, when properly inspected for the removal of prematurely ripe, cracked, or partially decayed fruits, offer no danger as carriers of the Mediterranean fruit fly, provided they are wrapped and shipped in accordance with the demands of the trade and the Federal regulations.

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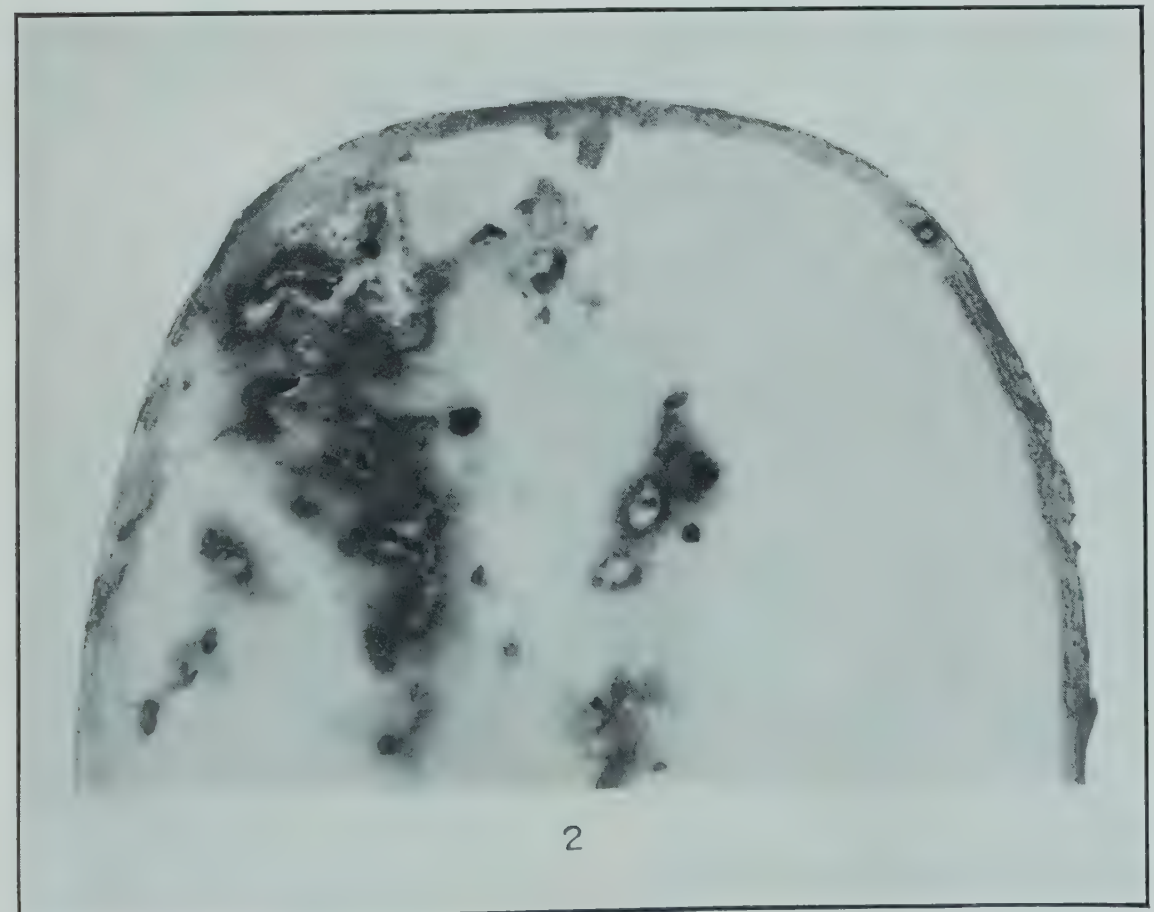
PLATE LIX

Fig. 1.—Popoulu variety of cooking banana found infested with the Mediterranean fruit fly. Note holes made in peel by the emerging larvæ. This fruit was fully ripe when found infested; mature fruits still green in color, present on the same bunch, were not infested.

Fig. 2.—Cross section of the Moa variety of cooking banana, showing pulp infested by larvæ of the Mediterranean fruit fly. Larvæ were found infesting the pulp of this variety only when the fruits had become mature, though not yellow in color, and when the peel had cracked sufficiently to expose the pulp, thus removing Nature's barrier to infestation.

1



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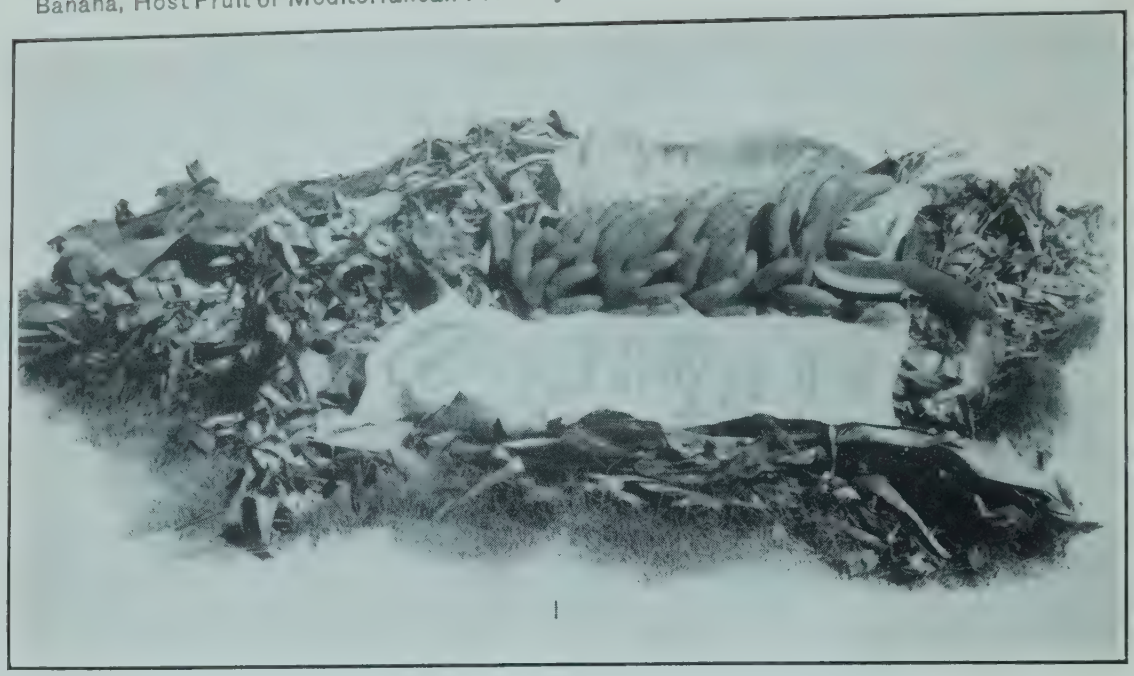

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EFFECT OF CONTROLLABLE VARIABLES UPON THE PENETRATION TEST FOR ASPHALTS AND ASPHALT CEMENTS

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INTRODUCTION

No one test for asphalts and asphalt cements is probably better known or more generally used than the penetration test. Many instruments have been devised for determining the consistency of these materials, but none have been generally adopted that do not substantially conform to the fundamental principles of the apparatus known as the Dow penetration machine.[1] This machine and others designed to give practically equivalent results are too well known to require description in this paper. In general, however, it may be said that by their use the consistency of asphalts or asphalt cements is expressed as the depth in hundredths of a centimeter that a standard needle will penetrate them vertically without external friction while the material is maintained at a stated temperature and the needle is operated under a stated load for a stated length of time. In the Dow penetration machine external friction is practically eliminated. In other satisfactory types it is reduced to an almost negligible minimum, but when operating with those in which the needle holder slides through a guiding sleeve it is most important that both the plunger and sleeve be absolutely clean and dry, as a small amount of moisture, oil, or dirt will produce considerable friction and thus retard the penetration of the needle into the sample being tested. Certain standards of temperature, load, and time have been generally adopted, and the most widely used combination is 25° C., 100 gm., 5 seconds.

Granting that the apparatus is mechanically satisfactory and that a definite standard needle is used, the test appears to be comparatively simple. It has frequently been found, however, that different laboratories, working upon samples of the same material under supposedly identical conditions of temperature, load, and time, obtain appreciably different results. The object of this investigation has therefore been to determine what effect apparently slight differences in these conditions will produce in the results of tests and also to study the importance of other controllable variables.

[1] Dow, A. W. The testing of bitumens for paving purposes. *In* Proc. Amer. Soc. Testing Materials, 6th Ann. Meeting 1903, v. 3, p. 349–368, fig. 1–6. Discussion, p. 369–373. 1903.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
cb

Vol. V, No. 17
Jan. 24, 1916
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The materials for this work were selected with the idea of obtaining products which showed rather wide differences in physical and chemical properties. For this purpose four types of oil asphalt were selected, which, being practically all bitumen, eliminated to a large extent variations due to sampling, which might have occurred in the case of native asphalts or fluxed native asphalts carrying appreciable quantities of non-bituminous material. The types represented in the following tables are produced from (1) steam-refined California petroleum, (2) steam-refined Mexican petroleum, (3) refined blended petroleum, and (4) blown petroleum. Three grades of each type were selected, having, at 25° C., under a load of 100 gm. applied for 5 seconds, penetrations of approximately 50, 100, and 150. This made 12 samples in all, and it is believed that the results obtained by their use can consistently be interpreted to cover practically all types of asphalts and asphalt cements. The more important physical and chemical characteristics of these products are shown in Table I.

TABLE I.—*Characteristics of asphalt cements*

Test.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Specific gravity, 25°/25° C.	1.039	1.036	1.026	1.048	1.046	1.036	1.026	1.025	1.031	0.993	0.988	0.987
Melting point (cube method)	53° C.	46° C.	42° C.	62° C.	52° C.	46° C.	62° C.	58° C.	44° C.	114° C.	82° C.	68° C.
Penetration, 25° C., 100 gm. 5 sec.	47	93	133	50	90	150	62	92	157	44	91	136
Penetration, 0° C., 200 gm., 1 min.	3	12	18	13	26	40	22	36	39	27	47	59
Penetration, 46° C., 50 gm., 5 sec.	Soft	Soft	Soft	227	Soft	Soft	220	310	Soft	70	176	305
Loss, 163° C., 20 gm., 5 hrs.	.77	.90	1.28	.09	.16	.46	.38	.87	.99	.14	.20	.20
Penetration residue, 25° C., 100 gm., 5 sec.	26	45	61	37	55	87	45	58	92	38	86	118
Bitumen-soluble (CS_2)	99.82	99.46	99.74	99.84	99.92	99.95	99.66	99.92	99.82	99.54	99.61	99.49
Organic insoluble	.06	.36	.20	.10	.06	.05	.21	.07	.13	.27	.21	.29
Inorganic insoluble	.12	.18	.06	.06	.02	.00	.13	.01	.05	.19	.18	.22
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Bitumen insoluble, 86° B. nap	21.4	19.9	18.0	29.8	29.5	25.7	29.1	28.9	25.7	29.5	24.8	22.7
Fixed carbon	13.4	13.3	11.0	18.1	17.6	15.3	14.0	14.2	14.5	13.3	12.4	11.3

The first consideration which naturally presents itself is the method of preparing the sample for the test. It is apparent that in order to duplicate results upon different samples of the same material the samples shall be taken so as to represent the entire body of material sampled.

It is assumed that in all instances laboratories take representative samples. The handling of the sample, once it is taken, however, is subject to a number of conditions which are not ordinarily strictly specified. In the first place, the sample must be melted by the application of heat and, to prevent any change during the melting process, it should be heated at as low a practicable working temperature as consistent with the time required to melt it. That is, all asphalts and asphalt cements tend to harden upon being heated, due either to loss by volatilization or to so-called oxidation or reaction with atmospheric air. This tendency is increased as both the temperature and time of melting are increased. The method followed in preparing all of the samples for this investigation was as follows:

About 6 ounces of each of the 12 materials were placed in pint tin cups. The 12 cups were then placed upon a ¼-inch asbestos board resting directly upon a gas hot plate. The samples were stirred occasionally to expedite melting, and removed from the hot plate as soon as completely fluid. At no time were the samples heated sufficiently to produce fuming. Upon removal from the hot plate the samples were poured into 3-ounce cylindrical tin dishes, measuring 5.5 cm. in diameter, with vertical sides approximately 3.5 cm. in height. While still fluid, all air bubbles which rose to the surface were removed by means of a tiny gas flame, which was rapidly passed over the surface and which merely caused the bubbles to break without in any way injuring the sample.

As the effect of the size of the container upon the results of tests had been investigated by Reeve,[1] it was felt that by the use of the dish above stated no danger of influencing results from this cause need be feared. In this connection it is of interest to note that Reeve's work demonstrated that a dish of 5 cm. or more in diameter could not influence the results of tests, although appreciable variations in results were in some cases caused by dishes smaller than 2.5 cm. in diameter.

EFFECT OF VARIATIONS IN METHOD OF PREPARING MELTED SAMPLES FOR TESTING

Undoubtedly the most common method of preparing a melted sample for the penetration test is to allow it to cool in air at room temperature for approximately an hour, then to immerse it for an hour in water maintained at the temperature at which the test is to be made. The sample is then tested under water at this temperature. In certain cases, cooling the sample in ice water or crushed ice prior to immersing it in the constant-temperature bath has been resorted to, and the penetrations so obtained have frequently been somewhat lower than those obtained by the method first described. As great a difference as 15 points in one asphalt cement

[1] Reeve, C. S. Effect of diameter of bitumen holder on the penetration test. *In* Proc. Internat. Assoc. Testing Materials (6th Cong. New York 1912), v. 2, no. 11, Paper 25_3, 4 p. 1912.

of about 150 penetration has been noted by the authors in this connection. The theory has been advanced that the ice-water cooling produces a set in the material which is not attained by the sample if it is allowed to air-cool until it has stood for a number of days. It has been further argued that the penetration at this set represents more accurately the true consistency of the material than does the penetration determined by the method first described. In order to study this matter thoroughly, different samples of each of the 12 materials were cooled and prepared for testing in a variety of ways, careful attention being paid to the time during which the sample was subjected to a given condition. These conditions are shown in Table II.

For each test under a given set of conditions samples of materials were melted and poured at the same time. In methods 1 to 6 and 15 to 23, inclusive, the melted samples were poured into the test dishes and, after standing in air for the periods indicated, were immersed in a water bath carefully maintained at 25° C. for the time selected, prior to determining their penetration. At the expiration of this time they were tested in the water bath. In methods 7 to 10, inclusive, the melted samples were poured into test dishes which had been previously packed in ice. Here they were allowed to remain until transferred to the 25° water bath. In methods 11 to 14, inclusive, the melted samples were first poured into the test dishes and allowed to cool in air as indicated, after which they were placed in an ice-water bath for definite periods of time and then immediately transferred to the 25° water bath. In methods 24 and 25, the melted samples were poured into test dishes packed in crushed ice and kept there for 1 hour. They were then removed and allowed to remain in air for 28 days, after which they were placed in the 25° water bath just prior to testing as indicated.

TABLE II.—*Individual penetration tests on asphalt cements, 100 gms., 5 seconds, 25° C.*

Method No.	Conditions before test.			California.									Mexican.									Blended.									Blown.								
	In air.	In ice.	In 25° C. bath.	8961			8962			8963			8948			8949			8950			8994			8995			8996			8956			8957			8958		
1	30 min.		30 min.	57	54	50	132	114	100	177	158	143	60	57	53	109	105	96	200	180	161	68	64	64	105	100	97	197	182	169	44	44	44	109	106	98	184	160	150
2	30 min.		1 hr.	49	47	47	95	93	91	138	134	132	52	51	51	95	93	91	151	152	148	65	64	63	97	96	95	162	162	160	43	44	44	97	96	94	142	144	138
3	30 min.		1½ hrs.	47	47	47	93	93	93	133	133	133	50	50	51	90	90	90	150	151	150	63	62	62	92	92	91	156	157	157	44	44	44	91	92	90	137	137	135
4	1 hr.		30 min.	51	49	48	100	96	92	145	141	135	53	51	50	94	93	93	159	156	149	65	62	61	98	97	94	165	161	159	43	44	44	98	97	93	143	142	141
5	1 hr.		1 hr.	47	46	46	94	94	93	131	130	129	51	50	49	93	90	90	150	150	149	63	62	62	94	93	93	158	156	156	42	43	42	93	92	91	137	138	138
6	1 hr.		1½ hrs.	46	46	46	95	95	95	133	134	134	50	50	49	90	91	89	148	147	146	61	62	60	93	93	92	158	159	156	41	42	43	91	91	91	136	136	136
7		30 min.	1 hr.	48	47	45	94	93	90	129	130	127	47	50	50	91	92	90	149	150	149	64	63	61	93	91	92	160	158	156	43	44	43	94	93	90	137	137	137
8		30 min.	1½ hrs.	47	47	46	93	93	93	130	131	131	49	49	49	91	90	89	151	151	150	61	62	60	93	94	94	158	157	155	43	43	43	92	92	89	135	135	136
9		1 hr.	1 hr.	46	46	46	93	93	92	129	129	129	49	50	48	88	87	86	146	146	144	61	60	61	94	93	92	156	154	151	42	42	43	92	90	92	134	134	136
10		1 hr.	1½ hrs.	46	46	48	93	94	94	133	133	132	49	49	50	88	87	86	147	147	145	59	60	60	93	92	91	154	152	150	41	42	42	90	90	92	137	137	135
11	30 min.	30 min.	1 hr.	49	46	45	92	92	92	131	128	128	50	49	48	90	89	88	148	152	150	61	61	61	92	93	92	155	155	153	41	43	42	90	91	92	135	136	137
12	30 min.	30 min.	1½ hrs.	46	47	46	93	93	92	132	132	132	49	49	48	88	89	87	147	147	145	60	60	60	93	93	92	155	153	152	43	43	42	90	91	92	136	135	135
13	30 min.	1 hr.	1 hr.	47	45	46	92	95	94	132	131	130	50	49	49	88	87	86	142	141	140	60	59	59	92	92	92	152	150	150	41	42	44	90	90	89	134	134	135
14	30 min.	1 hr.	1½ hrs.	47	48	47	92	92	92	133	133	132	48	48	47	87	87	87	143	143	140	59	60	59	92	92	91	151	150	148	42	43	43	92	92	91	134	135	134
15	24 hrs.		30 min.	44	43	43	94	93	92	133	131	130	46	47	48	86	86	86	144	144	142	60	60	58	89	89	88	151	151	150	42	41	40	88	89	88	130	130	130
16	24 hrs.		1 hr.	45	45	44	93	94	93	132	132	130	46	46	47	86	86	86	141	143	138	58	58	58	89	90	87	151	153	150	40	42	41	89	90	89	130	130	130
17	24 hrs.		1½ hrs.	44	45	45	93	93	93	131	132	131	46	47	47	87	86	86	142	142	141	58	58	58	88	89	88	150	151	150	40	41	43	89	89	89	132	132	132
18	3 days.		1 hr.	45	44	44	90	89	89	128	130	129	46	46	45	81	82	81	139	139	137	55	55	55	84	85	83	143	144	142	41	42	41	87	87	87	126	126	125
19	3 days.		1½ hrs.	44	44	44	91	90	90	130	129	130	45	45	45	83	83	82	140	140	138	54	54	54	86	85	84	144	145	143	42	42	41	89	88	88	128	127	128
20	7 days.		1 hr.	41	41	40	80	82	83	128	127	127	43	44	44	79	79	78	132	131	130	52	53	52	79	82	81	142	142	140	39	40	40	86	86	88	130	130	129
21	7 days.		1½ hrs.	43	43	42	85	85	85	125	126	124	44	45	45	78	79	78	131	131	130	53	54	53	82	82	81	142	142	141	41	42	42	85	85	86	128	128	129
22	28 days.		1 hr.	38	38	39	80	80	80	119	120	118	40	41	40	70	70	69	123	123	123	48	48	48	72	73	72	128	131	127	39	39	38	80	80	78	110	110	113
23	28 days.		1½ hrs.	38	38	38	79	79	78	118	119	119	38	39	39	70	70	69	123	124	124	48	48	48	73	74	73	132	132	129	38	38	37	79	78	78	112	112	112
24		1 hr.		36	36	37	79	80	80	118	119	118	39	39	39	70	68	68	123	123	124	48	48	49	72	72	73	127	128	126	38	38	38	77	77	74	113	112	112
	28 days.		1 hr.																																				
25		1 hr.		37	37	37	79	79	78	119	119	118	38	39	38	68	68	68	123	124	124	48	48	48	74	74	74	132	131	128	37	37	37	77	77	74	112	112	110
	28 days.		1½ hrs.																																				

Table II gives the results of three determinations for each sample under each of the conditions tried. These penetrations were all taken with the same needle at different points on the surface of the sample. Reading from left to right, the first test was made at the center, the third 1 cm. from the edge of the dish, and the second halfway between the positions of the first and third tests. For the dish measuring 5.5 cm. in diameter, the first penetration was therefore taken 2.7 cm., the second about 1.9 cm., and the third about 1 cm. from the edge of the dish.

It will be noted that the time elapsing between pouring the sample into the dish and determining its penetration varied from a total of 1 hour to over 28 days; that the immersion in the water bath directly preceding the test varied from 30 minutes to 1½ hours. Upon reviewing the results given in this table, it appears evident that, in general, for any given set of conditions preceding the immersion in the water bath, a 30-minute immersion in water gave less consistent check results than a corresponding 1-hour or 1½-hour immersion. Less difference is indicated between the 1-hour and 1½-hour immersions in water, but the balance of evidence appears to favor the latter period of time in so far as uniformity is concerned, even when negligible personal errors are taken into account. Thus, out of the 11 series of comparative tests of 1 hour and 1½ hours for all 12 materials, it will be found that in 61 cases the 1½-hour immersion gave the most consistent results; in 21 cases the most consistent results were obtained with the 1-hour immersion; and in 50 cases there is no preference so far as consistency in results was concerned.

If the average of the three tests for any sample is taken for the 1-hour air cooling and 1-hour immersion in the bath, as compared with the 30-minute air cooling and 1-½hour immersion in the bath, it will be found that they practically coincide. The fact, however, that in the latter case there is less difference between the individual results indicates that the 1½-hour immersion should have preference.

Eliminating the 30-minute immersion in the bath before making the test, and considering only the 1-hour and 1½-hour immersions in connection with short periods of prior cooling in air, Table III will be found to illustrate the differences above described. Here, comparing methods 5 and 3, it will be seen that in seven cases the most consistent results were obtained by the 1½-hour immersion; in two cases the 1-hour immersion produced the most consistent results; and in three cases there is no preference with regard to consistency in results. So far as rapidity in making the test is concerned, therefore, if a short-period air immersion is to be adopted, it would seem that 30 minutes in the air and 1½ hours in the bath prior to testing would be the most satisfactory minimum limits to adopt.

TABLE III.—*Comparison of penetration tests for short periods of air cooling and immersion in 25° C. bath, 100 gm., 5 seconds*

Method No.	Conditions before test.		California.									Mexican.									Blended.									Blown.								
	In air.	In 25° C. bath.	8961			8962			8963			8948			8949			8950			8994			8995			8996			8956			8957			8958		
2	30 minutes	1 hour	49	47	47	95	93	91	138	134	132	52	51	51	95	93	91	151	152	148	65	64	63	97	96	95	162	162	160	43	44	44	97	96	94	142	144	138
5	1 hour	1 hour	47	46	46	94	93	93	131	130	129	51	50	49	93	90	90	150	150	149	63	62	62	94	93	93	158	156	156	42	43	42	93	92	91	137	138	138
3	30 minutes	1½ hours	47	47	47	93	93	93	133	133	133	50	50	51	90	90	90	150	151	150	63	62	62	92	92	91	156	157	157	44	44	44	91	92	90	137	137	135

This being so, the average of results given in Table II can best be considered by means of Table IV, in which are given the average penetrations obtained on all of the samples under various conditions of cooling prior to 1½ hours' immersion in water. A study of this table shows in every case a gradual hardening or lowering of penetration as the time in air is increased. This lowering in penetration is not very pronounced in a period of 24 hours, but it increases quite appreciably in longer periods. Allowing for slight experimental errors, no difference is found to exist between the 30-minute and 1-hour exposure in air. The most marked difference is, of course, apparent between the results of 28 days in air as compared with 30 minutes in air, and the greatest difference in actual points of penetration will in every case, for a given type of material, be found for the softest grade of that type, or, in other words, for that grade which originally showed the highest penetration. It is apparent that no permanent set occurs up to a period of 28 days, but that a gradual hardening takes place. This being so, it is of interest to compare the foregoing with the results obtained by immersion in ice water prior to immersion in the water bath for 1½ hours at 25° C. It will be seen, in general, that but little difference in results is obtained between the samples cooled in ice water and those cooled in air, although under certain conditions for the short periods a slightly lower penetration has been secured by this means. It is safe to say, however, that the immersion of the sample in ice water does not produce a set which is comparable to any definite set produced by prolonged standing in air. This is evident from the last series of results, in which the samples which had been immersed in ice water for an hour were allowed to stand 28 days before immersing them in the water bath, the results in each case being appreciably lower than those obtained by immersing them for 1 hour in ice water and then 1½ hours in the bath just prior to test. There does not therefore, appear to be any good reason for cooling the sample in ice water at any time, except, perhaps, in plant-control work, where it is desired to expedite the test somewhat, and an allowance can be madefor variations from the ordinary method caused by the ice-water immersion.

TABLE IV.—*Comparison of average penetrations at 25° C. after 1½ hours' immersion in bath, 100 gm., 5 seconds*

Conditions before test.			California.			Mexican.			Blended.			Blown.		
In air.	In ice.	In air.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
.....	.....	30 min.	47	93	133	50	90	150	62	92	157	44	91	136
.....	.....	1 hr...	46	95	134	50	90	147	61	93	158	42	91	136
.....	.....	24 hrs.	45	93	131	47	86	142	58	88	150	41	89	132
.....	.....	3 days.	44	90	130	45	83	139	54	85	144	42	88	128
.....	.....	7 days.	43	85	125	45	78	131	53	82	142	42	85	128
.....	.....	28 days.	38	79	119	39	70	124	48	73	131	38	78	112
28 days (remelted).	.....	30 min.	46	95	133	49	91	148	61	89	156	42	93	136
.....	30 min.	.....	47	93	131	49	90	151	61	94	157	43	91	135
.....	1 hr...	.....	47	94	133	49	87	146	60	92	152	42	91	136
30 min....	30 min.	.....	46	93	132	49	88	146	60	93	153	43	91	135
30 min....	1 hr...	.....	47	92	133	48	87	142	59	92	150	43	92	134
.....	1 hr...	28 days.	37	79	119	38	68	124	48	74	131	37	76	111

Although all of the samples examined hardened very materially upon setting for 28 days, it is of interest to note that when these samples were remelted, allowed to cool in air for 30 minutes, immersed in the water bath at 25° C. for 1½ hours, and again tested, the penetrations, to all intents and purposes, were the same as those originally obtained by the 30-minute air cooling and 1½-hour immersion in the bath. This fact does not, however, indicate that the materials do not permanently harden with age, as Hubbard and Reeve [1] have shown that all types of bitumen permanently harden upon prolonged exposure.

As a result of the foregoing observations, the 30-minute air cooling and 1½-hour immersion in the bath prior to the test was adopted as the method of preparing samples prior to studying the effect of the variables, temperature, load, and time.

EFFECT OF VARIATIONS IN TEMPERATURE

The penetration of an asphalt cement is frequently determined and sometimes specified at three temperatures. The temperature most commonly employed and at which the consistency of the material is rated is 25° C. This is known as normal temperature, and the customary load and time factors used are 100 gm. and 5 seconds.

The penetration test is next frequently made at 0° C. with a load of 200 gm. applied for 1 minute. In some cases the test may be made with a load of 100 or 200 gm. applied for 5 seconds. For this test the sample is usually packed in finely crushed ice, which completely covers it, and the needle is brought in contact with its upper surface through a hole in the ice worked out with the finger. The needle itself, as well as the exposed surface, may, therefore, at the time of test be at a somewhat higher temperature than 0°. For this reason 4° C. has been selected by some for a low-temperature test, as it is a temperature which may be accurately maintained in the water bath.

Another temperature at which the penetration test is made is 46° C. Where possible, a load of 50 gm. is applied for 5 seconds, but in the case of materials which are very soft at this temperature the 50-gm. load is applied for 1 second.

In order to study the effect of variations in temperature upon the penetration test, a number of samples of each of the 12 asphalt cements were prepared, and after cooling in air for 30 minutes were placed for 1½ hours in the bath maintained at the test temperature. The results of these tests are given in Table V.

[1] Hubbard, Prévost, and Reeve, C. S. The effect of exposure on bitumens. *In* Jour. Indus. and Engin. Chem., v. 5, no. 1, p. 15–18, fig. 1–2. 1913.

TABLE V.—*Effect of variations in temperature on penetration of asphalt cements* [a]

Temperature.	Conditions at test.			California.			Mexican.			Blended.			Blown.		
	Load.	Time.	Bath.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
°C.	Gm.	Seconds.													
20.....	100	5	Water.	24	47	69	29	55	87	38	61	94	32	63	93
23.....	100	5	...do..	37	71	106	40	73	118	50	77	126	38	81	120
24.....	100	5	...do..	40	80	115	45	81	126	53	80	136	40	84	121
24.6.....	100	5	...do..	46	86	121	47	86	136	56	85	145	44	89	129
25.....	100	5	...do..	46	92	132	49	91	142	60	90	156	44	91	134
26.....	100	5	...do..	53	100	149	54	99	153	65	97	169	47	98	144
27.....	100	5	...do..	60	120	172	58	106	174	68	105	187	50	101	153
0.....	100	5	Ice....	1	10	13	10	16	23	8	11	13	11	17	20
0.....	100	5	Brine.	1	3	4	4	7	10	6	10	11	8	15	19
4.....	100	5	Water.	2	5	8	7	14	17	11	17	17	14	24	31
0.....	200	5	Ice....	10	13	18	13	26	30	13	20	24	16	28	37
0.....	200	5	Brine.	2	6	8	8	13	16	12	18	20	19	29	35
4.....	200	5	Water.	7	11	15	12	17	25	16	25	27	22	39	49
0.....	200	60	Ice....	13	17	23	28	36	39	20	37	41	28	50	62
0.....	200	60	Brine.	3	12	18	13	26	40	22	36	39	27	47	59
4.....	200	60	Water.	15	25	35	18	38	59	30	48	73	36	70	89
44.....	50	1	...do..	139	239	347	99	177	268	105	152	264	45	108	175
45.....	50	1	...do..	147	263	Soft.	108	188	290	118	161	281	49	116	185
46.....	50	1	...do..	180	318	Soft.	116	204	308	121	174	306	54	124	200
47.....	50	1	...do..	189	Soft.	Soft.	126	224	Soft.	130	190	Soft.	56	129	211
44.....	50	5	...do..	294	Soft.	Soft.	195	330	Soft.	190	277	Soft.	64	155	264
45.....	50	5	...do..	318	Soft.	Soft.	204	Soft.	Soft.	209	286	Soft.	67	165	285
46.....	50	5	...do..	Soft.	Soft.	Soft.	227	Soft.	Soft.	220	310	Soft.	70	176	305
47.....	50	5	...do..	Soft.	Soft.	Soft.	245	Soft.	Soft.	244	Soft.	Soft.	73	186	Soft.

[a] In this and succeeding tables it will be noted that at 25° C. under a load of 100 gm. applied for 5 seconds, sample 8950 shows a materially lower penetration than in Tables II, III, and IV. No satisfactory explanation has as yet been found for this variation, as the maximum difference of eight points is too large to be attributed to experimental error. Numerous checks have been made upon the later results, which were obtained about three months after the first determinations. It is possible that the material had undergone some change during that period.

Considering first those tests made with a 100-gm. load applied for 5 seconds at temperatures ranging from 20° to 27° C., it will be seen that a difference of 1 degree makes a very decided difference in the recorded penetrations. In fact, the difference in penetration for all but the blown products and the harder grades of the other types is quite marked between 24.6° and 25°. Allowing for experimental errors, this difference of 0.4° is, in the case of sample 8963, responsible for a difference of 10 points' penetration. In general, the softer the material the greater the difference for any type. As specifications for the penetration at 25° of asphalt cements are frequently limited to a variation of 10 points, it is at once apparent that the temperature of the bath should be carefully maintained at the exact temperature required, and that accurately calibrated thermometers, which may be read to tenths of a degree centigrade, be used for this purpose.

Considering any or all of the three sets of tests made at low temperatures it is evident that the ice method is inaccurate, inasmuch as it frequently gives a higher penetration than the corresponding result with the 4° bath. It is evident, therefore, that if the temperature of 0° is used, a brine bath which may be maintained at 0° should be employed. It is further of interest to note that marked differences in penetration for all of the types are obtained between the 0° brine test and the 4° water test. From this it is apparent that the 4° test should not, as has sometimes been done, be considered the practical equivalent of a 0° test.

With regard to penetration tests at relatively high temperatures, it is of interest to note the accentuated effect of slight variations in temperature for any given material. This is due to the fact that all of the materials are much softer at this temperature. Thus, for a 50-gm. load applied for 5 seconds a difference of 24 points' penetration for 1° C. (between 45° and 46° C.) is noted for sample 8995, while for a 100-gm. load applied for 5 seconds at 25° C. a maximum difference of 9 points' penetration for 1° (between 25° and 26° C.) is shown for the same material.

THE EFFECT OF VARIATIONS IN LOAD

The penetration of asphalt cements is most frequently determined under a load of 100 gm. Penetration machines are, however, designed so that the combined weight of needle and plunger is 50 gm. The 100-gm. load is then obtained by placing an additional 50-gm. weight upon the plunger. A 100-gm. weight may also be used with the machine, so that loads of 50, 100, 150, and 200 gm. are possible. All of these loads are occasionally used in making the penetration test. It is clear that any variation in weight due to carelessness in manufacture or to changes brought about by the replacement of the original needle will most seriously affect the smaller loads—that is, a difference of 1 gm. should produce proportionately a more marked effect where the 50-gm. load is employed than with heavier loads. A variation of 1 gm. is, of course, much larger than would ordinarily be expected to exist in different instruments, but as great a variation as this has been noted by the writers. In order to determine the effect of variation in load, penetration tests were made upon all of the 12 samples with 1-gm. variations from the 50- and 100-gm. loads, and in addition to this the penetrations at intermediate loads between 50 and 200 gm. were determined in order to ascertain just what effect would be produced in the penetration of different types of asphalt cements by changes in load when the penetrations were all made for 5 seconds at a temperature of 25° C. The results of these tests are given in Table VI.

TABLE VI.—*Effect of variations in load on penetration of asphalt cements, 25° C., 5 seconds*

Load.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Gm.												
49	31	59	89	32	60	96	40	60	106	26	53	83
50	32	60	90	32	61	97	40	60	106	26	54	83
51	32	61	91	33	62	98	40	60	106	26	54	83
60	35	67	98	36	67	110	43	63	120	29	63	94
75	40	76	112	41	77	123	48	75	133	36	72	111
90	43	85	123	45	85	134	54	84	147	39	82	124
99	46	91	132	48	90	140	60	90	155	41	89	134
100	46	92	132	49	90	142	60	90	155	42	90	134
101	46	92	132	49	91	142	60	90	156	43	90	135
125	51	101	146	54	101	159	65	101	173	51	105	157
150	59	113	160	60	113	178	76	113	192	58	118	182
200	68	134	178	72	129	211	90	134	218	72	153	231

Upon reviewing these results it will be noted that a variation of 1 gm. in no case produces an appreciable variation in results. In fact, the greatest variation is found to be one point penetration, and, in many cases, no difference in penetration is to be observed. It is therefore obvious that errors due to the calibration of the weights are practically negligible.

In connection with the series of tests for any individual material, it is of interest to note that within certain limits the increase in penetration is almost proportional to the increase in load. In other words, practically a straight-line curve may be obtained by plotting for any material the load against the corresponding penetration and connecting these points. If this is done the projection of the line to the axis representing increments of load will not hit this axis at its intersection with the axis representing increments of penetration. In general, it appears that blown asphalts possess less surface tension and adhesiveness than steam-distilled asphalts. The penetration of a blown asphalt therefore represents more nearly the actual distance which the needle enters the sample. In the case of steam-distilled asphalts the surface of the sample is markedly depressed by the needle, and probably proportionally greater retardation of its movement is produced by material which adheres to it.

It is of interest to note that a steam-distilled asphalt having a higher penetration than a blown asphalt at 25° C. under a load of 50 gm. applied for 5 seconds may have a lower penetration than the same blown asphalt at 25° under a load of 100 gm. applied for 5 seconds. For this reason the relative penetrations of different types of asphalt do not necessarily indicate their relative hardness.

As would naturally be supposed, in general, the greatest variations in penetrations due to variations in load are obtained upon the softer materials or those showing the highest penetration at any given load. The blown products, however, show more variation than do the other types. This is probably due to the fact that the effect of surface tension and adhesion is less pronounced with the blown products than with the steam-distilled products.

It was thought unnecessary to study the effect of variations in load at other temperatures and for other periods of time, as there was no reason to suppose that the results would be different in character from those given. The changes in time and temperature would merely change the penetration of the material and should give results comparable with those obtained upon softer or harder grades of the same type.

EFFECT OF VARIATIONS IN TIME

Penetration determinations are ordinarily made for a period of 5 seconds, especially where the 100-gm. load is employed. In the case of materials which are quite hard they may be made for a period of 1 min-

ute and usually under a load of 200 gm. This is done in most 0° or 4° C. tests. If a material is normally very soft or becomes very soft at 46° a 1-second test under a load of 50 gm. may be used. The time of test may be controlled by means of a swinging pendulum, a second clock, or metronome. The last is to be preferred because it leaves the eye free to watch the test itself and at the same time incurs less chance of error.

In order to determine the effect of variations in time upon the penetration test, samples of all 12 asphalt cements were prepared and tested at 25° C. under a load of 100 gm. applied for periods ranging from 1 to 10 seconds. The results of these tests are given in Table VII, in which every value recorded represents an average of a number of determinations made directly for the intervals of time stated.

TABLE VII.—*Effect of variations in time on penetration of asphalt cements, 25° C., 100 gm.*

Time.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Seconds.												
1	25	50	62	26	48	77	35	53	80	33	63	91
2	32	63	84	33	62	96	45	67	105	36	73	108
3	38	74	104	37	73	116	52	76	124	39	80	117
4	42	84	118	44	81	132	56	84	136	42	85	126
4½	45	88	126	45	84	140	58	88	148	43	88	131
5	47	93	132	48	89	145	60	91	155	44	90	135
5½	49	98	137	50	91	150	62	94	160	45	91	138
10	64	130	183	61	116	192	74	115	210	49	104	159

Upon reviewing these results it will be noted that for any material a greater number of points penetration is recorded for the first second than for any other one second. In general, upon the basis of a 5-second test it will be found that about 50 per cent of the penetration occurs during the first second for all but the blown type. With this type, owing probably to less surface tension and adhesion, considerably more than 50 per cent of the total 5-second penetration occurs during the first second. After the first second there is a decided tendency for the penetration to become less and less for each succeeding second. But with the softer grades of material a difference of one-half second from the 5-second test may make as much as 7 points difference in penetration. It is evident, therefore, that for accurate work in the 5-second test the time of penetration should be controlled to within less than half-second variations. From numerous tests it appears that if a metronome is used, the time of penetration may be controlled by any careful operator to within a maximum variation of one-fifth second from the selected time of test, and this is believed to be sufficient for all practical purposes.

SUMMARY AND CONCLUSIONS

For the sake of convenience, the more important conclusions regarding the method of making penetration tests, which have been reached as a result of this investigation, are summarized below.

(1) Melted samples should be cooled for not less than 2 hours prior to test, and should be tested upon the same day that they are melted, preferably after 2 or 3 hours.

(2) Samples should be maintained at the testing temperature for not less than 1 hour, and preferably for 1½ hours prior to test.

(3) Upon standing in the air, prepared samples show a decreasing penetration, but no definite end point or set is produced up to 28 days.

(4) In ordinary laboratory work there is no apparent advantage in cooling samples in ice or ice water prior to determining their penetration at higher temperatures. Cooling in ice water is therefore not recommended.

(5) Samples should be maintained and tested within 0.1° C. of the desired temperature for accurate work, as a variation in temperature of less than 0.5° in temperature may produce a decided difference in results.

(6) Tests at 4° are not the practical equivalent of properly made tests at 0°.

(7) When making tests at 0°, samples should not be packed in crushed ice, but should be immersed in a brine bath.

(8) The increase in penetration of a material determined under given conditions of temperature and time is, within certain limits, almost proportional to the increase in load. For the 100- and 200-gm. loads variations of as much as 1 gm. do not as a rule seriously affect determinations. It is, however, recommended that in all cases the load should not vary more than 0.2 gm. from that desired.

(9) In any test, proportionally the greatest number of points penetration is obtained during the first second. In the 5-second test approximately one-half of the total penetration is obtained during the first second. A variation of one-half second may, however, produce an appreciable variation in results.

(10) A carefully calibrated metronome is recommended for securing the proper time control.

(11) Aside from possible variations in needles, it is believed that variations in results obtained upon the same material by different laboratories are more probably due to unobserved variations in the methods of preparing the sample and to the control of temperature than to any other causes.

(12) It is believed that a study of the penetration of various types and grades of bituminous materials under a variety of conditions of temperature, load, and time may throw considerable light upon their other physical and chemical characteristics, and may serve as a possible means of identifying their origin and method of manufacture. The writers propose to continue work along this line.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., JANUARY 31, 1916 NO. 18

EFFECTS OF REFRIGERATION UPON THE LARVÆ OF TRICHINELLA SPIRALIS

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INTRODUCTION

Prior to recent investigations, the first of which were briefly reported in a short article which appeared about two years ago (Ransom, 1914), it had been generally accepted as an established fact that the larvæ of *Trichinella spiralis* are very resistant to cold and that they survive exposure to temperatures much below the freezing point of water. In the article referred to, however, it was shown that former ideas concerning the resistance of trichinæ to cold were erroneous, and that as a matter of fact low temperatures have a very pronounced effect upon the vitality of these parasites. As a precise knowledge of the effects of refrigeration upon trichinæ is of considerable importance, an extended investigation has been made, the results of which are recorded in the present paper.

HISTORICAL SUMMARY

The following summary covers all of the published reports of experimental work on the effects of cold upon trichinæ so far as they could be traced in the literature.

Leuckart (1863a, p. 120) states that trichinæ are in the highest degree resistant to cold. He exposed some trichinous meat outdoors during cold January weather (−16° to −20° R.; −4° to −13° F.; −20° to −25° C.) for three days and nights. After thawing the meat, he fed it to a rabbit, which died a month later and was found to be very heavily infested with trichinæ. In another publication (1866a, p. 91) Leuckart notes that the place in which this meat was kept was somewhat protected, and it may therefore be presumed that the temperature to which the meat was actually exposed was probably not as low as indicated by the figures given. Leuckart remarks, however, that the meat was solidly frozen throughout.

Fiedler (1864, p. 466) exposed the leg of a trichinous rabbit to an outdoor temperature of −15° to −17° R. (−1.75° to −6.25° F.; −18.75°

to −21.25° C.) from January 6, 5 p. m., to January 7, 8 a. m.—i. e., for 15 hours. Examined on a warm stage, the trichinæ showed no movement. Some of the meat was fed to two rabbits on January 7, and on February 7, a month later, the rabbits were killed. In one of them a few encysted trichinæ were found. On January 16 he fed two rabbits with some trichinous meat which had been cut in fine pieces and exposed for 18 hours to a temperature of −11° to −12° R. (7.25° to 5° F.; −13.75° to −15° C.). On February 14 the rabbits were killed and carefully examined. No trichinæ were found.

Rupprecht (1864a) exposed trichinous meat during one night to an outdoor temperature of −18° R. (−8.5° F.; −22.5° C.) and found that the vitality of the trichinæ was not affected.

Kühn (1865b), according to Leuckart (1866a, p. 91), found that trichinous meat kept in an ice chamber for 1½ months was still infectious and that the trichinæ had lost their vitality only after the meat had been kept for 2 months in the ice chamber, the temperature of which was not given.

Gibier and Bouley (1882a) exposed some trichinous ham for 4 hours to temperatures of −27° C. (−16.6° F.) and −20° C. (−4° F.). In the first case the interior temperature reached −20° C. (−4° F.) and in the second −15° C. (5° F.). All of the trichinæ were found to be dead. They showed no movement when warmed, and they stained in a few minutes with anilin blue, methyl-anilin violet, and picrocarminate of ammonia. Some of the meat which had been frozen was fed during 8 days to five birds, which when examined later showed no trichinæ in the intestine; nor had any been found in the feces. Trichinæ from portions of the ham which had not been frozen were active when warmed to 40° C. and remained transparent and colorless for several days in staining solutions. Five birds of the same kind and age as those to which the frozen meat had been fed were similarly fed with the ham which had not been frozen, and large numbers of trichinæ were afterwards found in the feces and intestines.

These experiments of Gibier and Bouley seemed to show pretty clearly the destructive effects of low temperatures upon trichinæ, but later Gibier (1889a) came to the opinion that the death of the parasites was to be explained on the ground that they had already suffered a reduction in vitality from the action of salt, and, hence, readily succumbed to freezing. This opinion was based on the results of an experiment in which he exposed small fragments of fresh trichinous pork for 2 hours to a temperature of −20° to −25° C. (−4° to −13° F.). The parasites, when afterwards examined on a warm stage, were found to have lost none of their activity.

From the foregoing it would appear that the usual statements found in articles relating to *Trichinella spiralis* as to the resistance of this parasite to low temperatures have their principal basis in Leuckart's

single experiment, to which may be added, as supplementary support, Fiedler's first experiment, Rupprecht's experiment, and Gibier's experiment, a total of four experiments. Kühn's experiment perhaps has been considered as affording additional supporting evidence. The results of Fiedler's second experiment do not offset the results of his other experiment, nor those of Leuckart's and Gibier's experiments, as the failure to get an infestation in the two rabbits which were fed meat exposed for 18 hours to a temperature of 7.25° to 5° F. might have been brought about by something else than low temperature. Likewise, the results of Gibier and Bouley, when compared with those of Leuckart, Fiedler, and Gibier, tend to show only that trichinae are sometimes killed when exposed for a short time to temperatures below zero. The later explanation by Gibier (1889) that the trichinæ used in these experiments had lost so much vitality on account of previous salting of the meat that they succumbed, whereas they would not have done so if the meat had been fresh, has been accepted by those authors who have mentioned Gibier and Bouley's work. It should be noted, however, that in the experiment upon which Gibier (1889) based his explanation of the results of the earlier experiments by himself and Bouley the meat was exposed for only 2 hours as compared with 4 hours in the earlier experiments.

So far as appears in the available literature, after the later experiments conducted by Gibier (1889), no further work on the effects of cold upon trichinæ was done until the investigations undertaken by the present writer, 25 years later, the first of which were recorded briefly in an article (Ransom, 1914) already mentioned.

A few additional data gathered in these investigations were given in a later paper (Ransom, 1915).

Recently Schmidt, Ponomarer, and Savelier (1915) have published a preliminary report of some investigations of the effects of cold upon trichinæ in which they state that a long series of experiments has led to the following results:

1. A temperature of 0° C. (32° F.) has no influence upon the vitality of encysted trichinæ, even though it acts during a period of 11 days.

2. A temperature of −6° C. (21.2° F.) is easily withstood by trichinæ during a period of 10 days, but they revive slowly.

3. A temperature of −9° C. (15.8° F.) is sometimes fatal, but not always. The results are not always the same; they are uncertain.

4. A temperature of −15 to −16° C. (5° to 3.2° F.) is always fatal; the trichinæ never revive.

Winn (1915) exposed some trichinous meat out of doors away from the sun in February, 1914, for 16 days, at an average mean temperature of −18.8° C. (−2° F.) with a minimum of −25° C. (−13° F.) and a maximum of −12.2° C. (10° F.). Nine guinea pigs were fed upon this meat, and none became infested.

EXPERIMENTAL WORK

DESCRIPTION OF EXPERIMENTS

The first experiment was carried out in Chicago in September, 1913. The carcass of a naturally infested trichinous rat killed on September 11 was inclosed in a tin can and kept in a refrigerator until September 16, when it was placed in a refrigerated compartment known as a "freezer" in one of the meat-packing establishments, where it remained for nearly 6 days—i. e., 5 days, 22 hours. During this time the temperature (as recorded by a thermometer not compared with a standardized thermometer), read once daily, varied from −3° to −10° F.[1] When removed, the rat carcass was allowed to thaw by exposure to ordinary room temperature, after which eight trichinæ were isolated by dissection. Examined in water on a warm stage, they were found to be shrunken and motionless. They were left in a moist chamber and again examined the following day, when they were found to be no longer shrunken, but exhibited no movement. Two more trichinæ, isolated from the meat the day after removal from the freezer, were also found to be inactive. A guinea pig was fed some of the meat from the rat carcass on September 25 and was found to be free from trichinæ when examined on October 25.

The failure to discover any evidence of life among the trichinæ isolated from the frozen rat carcass led to further experiments.

In experiment 2, a small piece of the diaphragm of another trichinous rat, after the carcass had been kept in an ice box for 11 days, was sealed in a vial and kept in a freezing mixture at a temperature of 4° to 10° F. for 30 minutes. No active trichinæ were found on examination after thawing. The rest of the carcass of the same rat was then inclosed in a tin can and placed in a freezer maintained at a temperature of 13° to 15° F., recorded by means of a thermometer (six readings daily), afterwards compared with a standardized thermometer (experiment 3). After nearly 2 days (45½ hours) the can was removed from the freezer. Trichinæ isolated by dissection soon after the meat had thawed and examined in water on a warm stage were found to be shrunken and motionless, but resumed their normal appearance and became active in 10 to 30 minutes.

In experiments 4, 5, and 6, pieces of diaphragm of an artifically infested rabbit were sealed in small vials and exposed to a temperature of −6° F. for 10, 20, and 30 minutes, respectively; none of the trichinæ isolated by dissection from the meat after thawing showed any activity, and guinea pigs fed with the meat failed to become infested.

In experiment 7 the carcass of a naturally infested rat was kept in a tin can in a freezer at 13° to 15° F. (six readings daily; thermometer

[1] Because of the practical bearing of the experiments upon the meat-packing industry, refrigeration temperatures are given according to the Fahrenheit scale, which is the only temperature scale in common use in the United States.

compared with a standardized thermometer) for a period of nearly five days. Trichinæ isolated by dissection showed slight activity on a warm stage.

The methods employed in experiments 8 to 127 and a general discussion of these experiments are given in the following pages, but it has been found expedient in order to save space to omit from the narrative statements of the results. These are later set forth in tabular form (Tables I, II).

In experiment 8, a leg of the rabbit referred to in experiments 4 to 6 was inclosed in a tin can and kept in a freezer at −2° F. for 43½ hours (thermometer not compared with a standardized thermometer; one reading daily). The next day after its removal from the freezer some of the meat was chopped in fine pieces and placed in the incubator (38° to 40° C.) in a beaker containing an artificial gastric juice (water; hydrochloric acid, about 0.35 per cent; and pepsin—exact quantity of pepsin used not recorded). Unfrozen meat from the same rabbit was similarly treated, using a portion of the same lot of digesting fluid. After incubating overnight, the sediment in the beakers was washed with several changes of water by decanting and settling. Trichinæ from the two lots of digested meat were then examined in water on a warm stage and the number of active and inactive individuals recorded. A guinea pig was fed some of the meat after it had thawed, and another guinea pig was fed some unfrozen meat from the same rabbit as a control, both being killed and examined for trichinæ after the lapse of a month.

Substantially the same methods of examination and feeding of test animals, with control examinations and feedings, were employed in experiments 9 to 22b. Meat from trichinous rats and rabbits was inclosed in tin cans, placed in freezers, which were maintained at various temperatures, and kept there for various periods. Portions of the meat were digested in artificial gastric juice and washed and examined as in experiment 8. Guinea pigs were used as test animals in experiments 9 to 15, white rats in experiments 16 to 22b.

In experiments 23 to 34 the carcass of a hog which had been artificially infested with trichinæ by feeding trichinous meat from various sources at intervals during a period of four months was hung in a freezer, the temperature of which was recorded by means of a thermometer (six readings daily) which had been compared with a standardized thermometer. The dressed carcass weighed about 150 pounds. The head was removed and kept unfrozen in a cooler to provide material for control examinations and feedings. From time to time portions of the carcass were removed for examination and test feedings. The same methods of examination were followed as in experiment 8. Test animals, usually white or hooded rats, were fed, and one lot of rats was fed unfrozen meat from the same carcass as a control.

In experiments 35 to 48 the carcass of another hog artificially infested as in the case of the hog used in experiments 23 to 34, weighing about 125 pounds dressed, was split in halves, which were hung in two freezers kept at different temperatures. The same procedure as to examination and feeding of test animals was followed as in experiments 23 to 34.

In experiments 49 and 50 digested meat from a trichinous rabbit, after washing and sedimenting with water, was inclosed in small vials, frozen by immersion in a freezing mixture, and the trichinæ, after thawing, examined on a warm stage.

In experiments 51 to 55, a hog artificially infested as in experiments 23 to 48 was slaughtered, and meat from the carcass inclosed in five 1-pound cans which were placed in the center of five barrels 28 inches high by 17 inches in diameter at the ends and 20 inches in diameter at the middle, each containing about 250 pounds of pork trimmings. The head of the carcass was kept unfrozen in a cooler to provide material for control examinations and feedings. The barrels were placed in a freezer the temperature of which was recorded six times daily by means of a thermometer which had been compared with a standardized thermometer. The barrels were removed from the freezer after 7, 8, 9, 10, and 11 days, respectively, and allowed to thaw sufficiently to permit the removal of the cans of trichinous meat. Examinations of the meat were made as in experiment 8. White or hooded rats in lots of five or six were fed some of the meat on several successive days, a separate lot being fed from each can.

In connection with experiments 51 to 55, it may be noted that in another experiment it was found that the interior temperature (determined by an electrical thermometer) of a barrel containing 250 pounds of pork trimmings did not fall to the temperature of the freezer (5° to 7° F.) from an initial temperature of 32° until the barrel had been in the freezer for eight days.

In experiments 56 to 64 the carcass of the hog from which meat was taken for use in experiments 51 to 55 was hung in the same freezer, and portions were removed from time to time for examination and feeding of test animals, following the same procedure as in those experiments.

In experiments 1 to 64, specially reared white or hooded rats were used as test animals whenever possible, but in some cases it was necessary, on account of the lack of a sufficient supply, to utilize rats whose previous history was not fully known; and in other cases the use of guinea pigs was necessary. In the remaining experiments, 65 to 127, only white or hooded rats were used which had been specially reared for the purpose on food from which there was no possibility of acquiring an accidental infection with trichinæ.

The meat from six hogs was used in experiments 65 and 65a. Four of these were artificially infested hogs which had been fed with trichinous pork several months before they were slaughtered, in October, 1914.

The two others slaughtered about the same time were naturally infested, having been found trichinous on microscopic examination. A shoulder was taken from each carcass and kept unfrozen in a cooler to provide material for control examinations and feedings.

In experiment 65, trimmings were taken from each of the six carcasses and a quantity weighing 106 pounds was inclosed in a wooden box measuring 28 by 19 by 6½ inches. The box was placed in a freezer, where it remained for 19 days, the temperature of the freezer being recorded three times daily by a thermometer which was afterwards compared with a standardized thermometer. After removal from the freezer the box was allowed to thaw for 2 days. A portion of the meat was then taken from the middle, passed twice through a meat chopper, and digested and examined as in experiment 8, a control examination being made of a mixture of unfrozen meat from the same carcasses similarly prepared and digested. A definite formula was followed in the preparation of the digesting fluid, which was mixed in the following proportions: Water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm. Five rats were fed some of the ground meat, 50 gm. of which were placed in their cage on each of three days, a total of 150 gm., an average of 30 gm. per rat. As controls five rats were fed once an average of 10 gm. of unfrozen meat from one of the hog carcasses, another lot of five, 10 gm. each from another carcass, and so on—i. e., 30 rats in all, 5 for each hog.

In experiment 65a, some of the same lot of frozen trimmings were used and were examined and fed to five rats, following the same methods as in experiment 65. In this case the trimmings had been made into sausage meat after thawing, a curing mixture having been mixed with the meat, containing salt equivalent to 3⅓ per cent of the weight of the meat. After the addition of the curing mixture and until it was prepared for artificial digestion and feeding of test animals, the meat remained for two days in a cooler at a temperature of 36° to 37° F. Analysis showed that the meat contained 3.12 per cent of salt. In preparing it for examination and feeding tests, the meat, immediately after it was ground up, was washed in water to remove the salt.

In experiment 66, 8 pounds of meat from a naturally infested hog were inclosed in a box 15¾ by 9 by 3 inches and placed in a freezer the temperature of which was recorded three times daily by means of a thermometer which was afterwards compared with a standardized thermometer. After 19 days the box was removed and some of the meat was examined and fed to test animals, following the methods used in experiment 65. As controls, five rats were fed 50 gm. of unfrozen meat from the same carcass, an average of 10 gm. per rat.

In experiments 67 to 71, meat was taken from the same carcasses as that used in experiment 65. Mixed meat from the six hogs was placed in five half-pound tin cans. Each can contained an approximately

equal quantity of meat from each hog. Two of the cans were placed in freezers, one maintained at −9° to 0° F. (three readings daily; thermometer not compared with a standardized thermometer), the other maintained at 10° to 12° (three readings daily; thermometer compared with a standardized thermometer). When removed from the freezers, the cans were thawed at room temperature, the thawing of the meat from the can taken from the second freezer (10° to 12°) being hastened by pulling the pieces of meat apart (experiment 71). The examination and the feeding of test animals were carried out in the same manner as in experiment 65. The three other cans were placed in the center of boxes 28 by 19 by 6½ inches, each containing about 100 pounds of pork trimmings. These boxes were placed in the same two freezers as the loose cans, two in the freezer maintained at the lower temperature (experiments 67, 68), the third box in the other freezer (experiment 70). When removed from the freezer, the boxes were allowed to thaw for two days. The cans were then removed and the meat examined and fed to rats, following the methods used in experiment 65.

In experiments 72 to 76 meat was taken from an artificially infested hog which had been fed trichinous meat several months prior to its slaughter in November, 1914, and this meat was inclosed in five half-pound tin cans. A ham from the carcass was kept unfrozen, at first in a cooler and afterwards in an ice box, to provide material for control examinations and feedings. Two of the cans were placed in a freezer maintained at a temperature of −9° to 2° F. (three readings daily; thermometer not compared with a standardized thermometer), two in a freezer maintained at a temperature of 10° to 13° (three readings daily; thermometer compared with a standardized thermometer), and the fifth in the center of a box 28 by 19 by 6½ inches, containing about 100 pounds of pork trimmings, this box being placed in one of the freezers (−9° to 2°) just mentioned.

The meat in the loose cans was allowed to thaw rapidly when removed from the freezers; that in the box required two days to thaw so that the can could be readily removed. The same methods of examination were followed as in experiment 65, except that some of the examinations were made in a 0.6 per cent salt (sodium chlorid) solution following digestion of the meat, the digested meat in those cases being washed with a 0.6 per cent salt solution instead of water. The use of a 0.6 per cent salt solution was adopted when it was discovered that trichinæ digested out of meat commonly become inactive if kept from a half an hour to several hours in water at a temperature of 32° to 40° C. This does not occur in cold water nor in warm salt solution. In the earlier experiments the use of plain water probably led to no misleading results, however, as every examination was controlled by an examination of unfrozen meat similarly treated. The same methods with reference to the feeding of test animals were followed in experiments 72 to 76 as

in No. 65. Four rats as controls were fed a total of 20 gm. of meat on July 8, 1915, from the ham which had been kept unfrozen since the slaughter of the hog—nearly eight months. No infections resulted. The trichinæ had evidently died. Examination on August 25 of some of the meat after artificial digestion showed only a few trichinæ. These were dead and disintegrated. There is little doubt, however, that if control animals had been fed early enough, they would have become infested, since trichinæ from the unfrozen meat examined after artificial digestion as late as three weeks after slaughter of the hog were quite lively and appeared altogether normal.

In experiments 77 to 87, meat from five trichinous hogs was used. Three 1-pound cans (5½ by 2¾ inches) were filled with meat from the first hog. One of the cans was placed in the center of a box 28 by 19 by 6½ inches, containing about 100 pounds of pork trimmings, and another in the center of a barrel of pork trimmings weighing 383 pounds net (dimensions of the barrel not recorded). Two cans were filled with meat from the second hog and two each in the case of the third, fourth, and fifth hogs, and one can of meat from each hog was placed in the center of a box of trimmings, as was done with one of the cans of meat from the first hog. A shoulder from each hog was kept unfrozen to provide material for control examinations. These shoulders were kept in a cooler or an ice box, except during the time when they were in transit between Chicago and the Washington laboratory.

The five boxes and the barrel were placed in a refrigerated compartment or freezer, maintained at a temperature of −2° to 5° F. The five loose cans were placed in a freezer maintained at 12° to 16°. The boxes were kept in the freezer for 15 days, the barrel for 23 days, and the loose cans for 17 days. During the time the meat was in the freezers the temperature was recorded three times daily, using a thermometer which was afterwards compared with a standardized thermometer, and found to be substantially correct. The temperature of the freezer in which the boxes and the barrel were kept varied from −2° to 5° during the time the box and barrel containing meat from the first hog were in it. During the time the four other boxes were in this freezer the temperature varied from −2° to 2°. The temperature of the freezer in which the five loose cans were kept varied between 12° and 16° during the time the can of meat from the first hog was in it, and between 13° and 15° during the time the four other cans were in it.

When the boxes were removed from the freezers after 15 days' exposure to cold, they were allowed to thaw slowly until the cans could be removed, which required two days (three days in one case, experiment 77). The thawing of the barrel required five days. After removal the cans were forwarded by mail from Chicago to Washington, where they were kept after arrival in an ice box or in a cooler (temperature, above 32° F.) until they could be examined. The time elapsing between removal from the

freezer and the placing of the meat in artificial gastric juice in preparation for examination varied between 6 and 12 days.

In preparing the meat for examination and feeding tests, the contents of the can were passed twice through a meat chopper, thoroughly mixing the ground meat together. Fifty gm. of ground meat from each can were placed in a beaker containing 600 c. c. of a freshly prepared artificial gastric juice made by the following formula: Water 1,000 c. c., hydrochloric acid (sp. gr. 1.19) 10 c. c., scale pepsin (U. S. P.) 2.5 gm. (experiments 77, 78); or the same formula modified by the addition of 6 gm. of sodium chlorid (experiments 79 to 87). The contents of the beaker were then stirred and carefully warmed to 40° C. and the beaker placed in an incubator (37° to 40° C.) for 18 to 24 hours. After removal from the incubator the supernatant fluid was decanted off, salt solution (0.6 per cent) added, the contents of the beaker stirred, allowed to settle, again decanted, more salt solution added, and so forth, until the supernatant fluid remained clear and transparent. As a control upon a possibly injurious effect of the digestant on the trichinæ, 50 gm. of ground unfrozen meat from the same carcasses as the frozen meat to be examined were placed in 600 c. c. of the same lot of digestant prepared for digesting the meat which had been frozen, put into the incubator, and removed at the same time as the other, washed in the same manner, and handled in all respects exactly the same as the meat which had been frozen. The sediment which remained in the beakers after washing and decanting was examined in salt solution (0.6 per cent) on a warm stage under the microscope.

In the tests on animals five white or hooded rats, reared from birth on food from which there was no possibility of acquiring an accidental infection with trichinæ, were used for testing each lot of meat. The five rats were kept together in a cage and 50 gm. of the ground meat were placed in the cage each day for three days, a total of 150 gm. of meat, or an average of 30 gm. per rat. The cage was watched to see that the meat was all eaten. It was usually eaten promptly. The rats which died within the first two weeks were examined for the presence of trichinæ in the intestine as well as in the muscles. In the case of those which died later only the diaphragm was examined. A month or more after feeding, the surviving rats were killed, and their diaphragms were examined. Through an oversight no control animals were fed with unfrozen meat from the five hogs from which the meat was obtained for use in this set of experiments (77 to 87). In view of the undoubted viability of the trichinæ in these hogs, however, as determined by the fact that the trichinæ obtained from digested unfrozen meat were practically all active, very lively, and quite normal in all respects, this omission is not of great importance.

In the next series of experiments (88 to 90), meat was taken from the shoulders of seven naturally infested hogs slaughtered during December,

1914, and was inclosed on January 17, 1915, in three 1-pound cans (5½ by 2¾ inches), each can containing meat from all seven hogs. The shoulders after slaughter of the hogs were kept in a cooler at a temperature a few degrees above 32° F., except during the time when they were in transit between Chicago and Washington. Five of the seven hogs were the same as those from which the meat for experiments 77 to 87 was taken. On January 18 the three cans were placed in three freezers in New York City where they remained until February 1, a period of 14 days or, to be exact, 13 days, 23 hours. The temperature of the freezers as determined by thermometers compared with a standard thermometer during this period was 4° to 7°, 8° to 11°, and 14° to 16° F., respectively (four readings daily). After removal from the freezers the cans were allowed to thaw at ordinary temperatures and were received for examination at the Washington laboratory on February 4.

The same routine as to the examination and feeding of experimental animals was followed as in the preceding experiments (77 to 87) except that the digesting fluid used contained only 5 gm. of sodium chlorid to each 1,000 c. c. of water, instead of 6 gm. In this case, as in the preceding set of experiments, no control animals were fed, but it happened that the test animals fed with the meat exposed to the temperature of 14° to 16° F. became infested, so that they served as a control upon those fed with meat exposed to the lower temperatures.

In the series of experiments numbered 91 to 126, the meat used was taken from six hogs slaughtered in Chicago prior to March 2, 1915, and found to be trichinous on microscopic examination. A shoulder from each of these hogs was sent in the fresh condition to Washington where it was retained in a cooler slightly above 32° F. to provide material for control examinations and feedings. The meat for the freezing experiments was inclosed in thirty-six 1-pound tin cans (5½ by 2¾ inches), some from each of the 6 hogs being placed in each can, so that each can contained a mixture of approximately equal portions of meat from all the hogs. On March 2, twelve of the cans were placed in a freezer maintained at a temperature of about 5° (5° to 6.5°), 12 in a freezer maintained at a temperature of about 10° (9° to 13°), and 12 in a freezer maintained at a temperature of about 15° (13.5 to 15°). After 10 days—on March 12—a can was removed from each of the 3 freezers and sent by mail to the Washington laboratory. The next day 3 more cans were removed as before, and so forth, the last cans being removed on March 25, after 23 days' exposure to cold. None was removed March 14 or 21, or 12 and 19 days, respectively, after they were placed in the freezers. The thermometers in these freezers, which were afterwards compared with a standardized thermometer, were read three times daily.

The same routine examination was followed as in experiments 77 to 90, described above, the formula of the digestant fluid being that used

in experiments 78 to 90—i. e., water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm.; sodium chlorid, 5 gm. A mixture of unfrozen meat from the six hogs was used in control examinations. As in the preceding experiments, five rats were fed meat from each can, following the same routine. Control animals were fed on June 15 with unfrozen meat from the six hogs which had been kept several months (since March) in a cooler. Meat from each hog was fed to two rats, 20 gm. being given to each two rats, an average of 10 gm. per rat.

In experiment 127, some meat from an artificially infested hog (the same hog from which meat was obtained in experiments 72 to 76) was inclosed in a half-pound tin can, which was placed in the center of a box 28 by 19 by 6½ inches containing about 100 pounds of pork trimmings. The box was placed in a freezer in Chicago, where it remained for 57 days, during which time the temperature as recorded by a thermometer afterwards compared with a standardized thermometer varied between 10° and 13° F. (three readings daily). After removal from the freezer the box was allowed to thaw for two days. The can was then removed and sent to the Washington laboratory. The same routine as to the examination and feeding of test animals was followed as in experiments 91 to 126.

There were no satisfactory control test animals in experiment 127, as the rats fed as controls in experiments 72 to 76, which would have served as controls in this experiment, were not fed until nearly eight months had elapsed since the slaughter of the hog from which the meat was obtained. No infestation resulted in these animals; the trichinæ were evidently all dead. Examination of some of the meat about six weeks later showed that the trichinæ were dead and disintegrated. The trichinæ, however, that were examined after artificial digestion of unfrozen meat from this hog as late as three weeks after slaughter appeared perfectly normal and were quite lively, and there is little doubt that control animals would have been infested if they had been fed early enough.

See Tables I and II for the results of these experiments.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)[a]	(g)	(h)[a]	(i)	(k)	(l)	(m)	
			°F.										
1	1 rat	Carcass	−3 to −10	6	10	0	.....	.....	0	1	.....	.....	(k) Guinea pig.
2	...do	Vial	4 to 10	(b)	10	0	.....	.....	.....	.....	.....	.....	
3	...do	Carcass	13 to 15	2	12	100	.....	.....	.....	.....	.....	.....	
4	1 rabbit	Vial	−6	(c)	4	0	.....	.....	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
5	...do	.....do	−6	(d)	2	0	.....	.....	0	1	1	0	Do.
6	...do	.....do	−6	(b)	.....	.....	.....	.....	0	1	1	0	Do.
7	1 rat	Carcass	13 to 15	5	2	100	.....	.....	.....	.....	.....	.....	
8	1 rabbit	Leg	−2	2	184	24	41	95	0	1	1	0	(k, l) Guinea pigs; (l) slightly infested.
9	...do	.....do	−2 to +3	3	299	2	.....	.....	0	1	1	0	(f) Mostly sluggish; (k) guinea pig; (l, m) same as No. 8.
10	...do	Part of carcass.	−2 to +3	5	606	0	104	100	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
11	...do	.....do	11 to 15	6	80	81	104	100	1	0	1	0	(f) Nearly all very lively; (g, h) same as No. 10; (i) guinea pig slightly infested; (l, m) same as No. 8.
12	...do	.....do	−2 to +3	6	275	1−	(?)	100	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
13	...do	.....do	−1 to −2	5	433	0	37	100	0	1	1	0	Do.
14	...do	.....do	−2 to +3	8	200	0	37	100	0	1	1	0	(g, h) Same as No. 13; (k) guinea pig; (l, m) same as No. 8.
15	...do	.....do	13 to 15	12	64	0	26	100	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
16	...do	.....do	4 to 14	5	25	0	152	14	0	1	1	0	(g) In water 4 days after digestion.
17	...do	.....do	12 to 14	5	100	7	152	14	0	1	1	0	(e) In water 4 days after digestion; (f) 4 out of 7 very sluggish; (g, h, l, m) same as No. 16.
18	...do	.....do	14 to 22	5	42	5	152	14	0	1	1	0	(e) In water 3 days after digestion; (f) very sluggish; (g, h, l, m) same as No. 16.
19	1 rat	.....do	4 to 14	5	70	0	20	100	0	1	1	0	
20	...do	.....do	12 to 14	5	73	14	20	100	0	1	1	0	(g, h, l, m) Same as No. 19.
21	1 rabbit	.....do	4 to 14	6	.....	.....	.....	.....	0	2	1	0	(l, m) Same as No. 16.
21a	...do	.....do	12 to 14	6	.....	.....	.....	.....	0	2	1	0	Do.

[a] Percentages in columns (f) and (h) are expressed in the nearest whole numbers, except that percentages over 99.5 are expressed as 99 + and less than 0.5 as 1−.
[b] 30 minutes. [c] 10 minutes. [d] 20 minutes.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
22	1 rat	Part of carcass.	14 to 22	10	58	31	168	100	0	1	1	0	(f) Very sluggish; (l, m) same as No. 19.
22a	1 rabbit	do	14 to 22	10	.....	.....	.....	.....	0	2	1	0	(l, m) Same as No. 16.
22b	do	do	12 to 14	10	.....	.....	.....	.....	0	2	1	0	Do.
23	1 hog	Carcass	14.5 to 15.5	7	476	98	208	97	6	0	2	1	(i) Four heavily, 2 moderately infested; (l, m) same as No. 34.
24	do	do	14.5 to 15.5	9	216	99+	120	100	6	0	2	1	(i) Four heavily infested; 1 degree of infestation not recorded; 1 guinea pig slightly infested; (l, m) same as No. 34.
25	do	do	14.5 to 16.5	10	180	59	182	98	0	4	2	1	(f) Sixty-three out of 107 sluggish; (k) 6 fed, 1 died after first feeding, another lost from cage; meat (loin) fed very lightly infested; (l, m) same as No. 34.
26	do	do	14.5 to 16.5	11	273	76	63	87	2	4	2	1	(f) One hundred and forty-five out of 208 sluggish; (i) slightly infested; (l, m) same as No. 34.
27	do	do	14.5 to 16.5	12	107	95	61	99	0	6	2	1	(f) Fifty-seven out of 102 sluggish; (l, m) same as No. 34.
28	do	do	14.5 to 16.5	13	59	42	61	99	0	6	2	1	(f) Twenty out of 25 sluggish; (g, h) same as No. 27; (l, m) same as No. 34.
29	do	do	14.5 to 16.5	14	.....	.....	.....	.....	6	0	2	1	(i) Three moderately, 2 slightly, 1 heavily infested; (l, m) same as No. 34.
30	do	do	14.5 to 16.5	16	102	74	99	99	2	1	2	1	(i) Slightly infested; (l, m) same as No. 34.
31	do	do	14.5 to 16.5	18	122	99	99	99	2	1	2	1	(g, h) Same as No. 30; (i) slightly infested; (l, m) same as No. 34.
32	do	do	14.5 to 16.5	21	135	18	123	80	0	3	2	1	(e) Meat digested 2 days; (g) meat from another carcass digested 2 days; (l, m) same as No. 34.
33	do	do	14.5 to 16.5	22	110	84	200	100	0	3	2	1	(f) Fifty-three out of 92 sluggish; (g) meat from another carcass; (k) guinea pigs; (l, m) same as No. 34.
34	do	do	14.5 to 16.5	24	.....	.....	.....	.....	0	3	2	1	(l) Heavily infested.
35	do	Half of carcass.	8.5 to 9.5	5	267	16	80	100	1	2	2	1	(i, k) Guinea pigs; (i) lightly infested; (l, m) same as No. 41.
36	do	do	8.5 to 9.5	6	452	2	75	100	0	3	2	1	(f) Sluggish; (k) guinea pigs; (l, m) same as No. 41.

37	...do.....	.....do.....	8.5	to	10.5	7	392	4	100	100	0	3	2	1	(f) Very sluggish; (l, m) same as in No. 41.
38	...do.....	.....do.....	8.5	to	10.5	8	298	0	123	80	.....	.....	.....	.....	(e, g) Meat digested 2 days.
39	...do.....	.....do.....	8.5	to	10.5	9	731	1—	200	100	0	3	2	0	(f) Very sluggish; (l, m) same as No. 41.
40	...do.....	.....do.....	8.5	to	10.5	10	701	1—	200	100	0	3	2	0	(f) One out of 3 sluggish, 2 very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
41	...do.....	.....do.....	8.5	to	10.5	11	.....	.....	.....	.....	0	3	2	0	
42	...do.....	.....do.....	5	to	7	5	221	14	80	100	3	0	2	0	(g, h) Same as No. 35; (i) 2 moderately, 1 slightly infested; (l, m) same as No. 41.
43	...do.....	.....do.....	5	to	8	6	159	0	75	100	0	3	2	0	(g, h) Same as No. 36; (k) guinea pigs; (l, m) same as No. 41.
44	...do.....	.....do.....	5	to	8	7	449	1—	100	100	0	3	2	0	(f) Very sluggish; (g, h) same as No. 37; (l, m) same as No. 41.
45	...do.....	.....do.....	5	to	8	8	326	0	123	80	.....	.....	.....	.....	(e) Meat digested 2 days; (g, h) same as No. 38.
46	...do.....	.....do.....	5	to	8	9	603	1—	200	100	0	3	2	0	(f) Very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
47	...do.....	.....do.....	5	to	8	10	517	0	200	100	0	3	2	0	(g, h) Same as No. 39; (l, m) same as No. 41.
48	...do.....	.....do.....	5	to	8	11	.....	.....	.....	.....	1	2	2	0	(i) Old infection, encysted trichinæ in diaphragm; rat died 7 days after first feeding; (l, m) same as No. 41.
49	1 rabbit.	Vial.....	7	to	10	(a)	354	0	37	100	.....	.....	.....	.....	(b) Sediment of digested meat in small vial.
50	...do.....	.....do.....	10	to	18	(b)	230	3	37	100	.....	.....	.....	.....	(b) Sediment of digested meat in small vial; (f) sluggish; (g, h) same as No. 49.
51	1 hog....	250 pounds..	10	to	12	7	12	100	100	100	5	0	.....	.....	(i) Heavily infested.
52	...do.....	.....do.....	9	to	12	8	.....	.....	.....	.....	6	0	.....	.....	Do.
53	...do.....	.....do.....	9	to	12	9	100	95	29	93	6	0	.....	.....	Do.
54	...do.....	.....do.....	9	to	12	10	21	90	29	93	5	1	.....	.....	(i) Heavily infested; (g, h) same as No. 53; (k) died 3 days after first feeding.
55	...do.....	.....do.....	9	to	12	11	62	71	21	100	6	0	.....	.....	(i) Heavily infested.
56	...do.....	Carcass.....	10	to	11	6	400	46	500	99	6	0	.....	.....	(i) Three heavily infested, 3 died early, few trichinæ found.
57	...do.....	.....do.....	10	to	12	7	.....	.....	.....	.....	5	0	.....	.....	(i) Heavily infested, one very heavily infested.
58	...do.....	.....do.....	9	to	12	8	105	71	100	100	4	0	.....	.....	(g, h) Same as No. 51; (i) 5 rats fed, 1 died, eaten by others, 1 slightly infested, 2 heavily infested, 1 number of trichinæ not recorded.
59	...do.....	.....do.....	9	to	12	9	.....	.....	.....	.....	5	1	.....	.....	(i) Slightly infested; (k) died 4 days after first feeding.
60	...do.....	.....do.....	9	to	12	10	.....	.....	.....	.....	6	0	.....	.....	(i) Heavily infested.
61	...do.....	.....do.....	9	to	12	11	.....	.....	.....	.....	6	0	.....	.....	(i) Three heavily, 3 moderately infested.
62	...do.....	.....do.....	9	to	12	12	48	83	29	93	5	1	.....	.....	(g, h) Same as No. 53; (i) 3 heavily, 1 slightly infested, 1 number of trichinæ not recorded; (k) died 1 day after first feeding.
63	...do.....	.....do.....	9	to	12	13	9	11	21	100	2	4	.....	.....	(g, h) Same as No. 55; (i) 1 trichina in each; (k) 1 died 4 days after first feeding.
64	...do.....	.....do.....	9	to	12	14	.....	.....	.....	.....	0	6	.....	.....	
65	6 hogs...	100 pounds..	10	to	13	19	523	31	127	98	4	1	28	1	(i) Very slightly infested; (l) all but 1 heavily infested.
65a	...do.....	.....do.....	10	to	13	19	578	3	195	98	2	2	28	1	(b) Meat after thawing cured with salt 2 days before examination and feeding; (i) very slightly infested, 4 trichinæ in each; (i, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
66	1 hog....	8 pounds....	11	to	13	19	105	100	116	98	0	4	5	0	(k) Five rats fed, 1 lost from cage; (l) 2 heavily, 3 slightly infested.
67	6 hogs...	100 pounds..	— 4	to	0	5	121	100	50	100	4	0	28	1	(i) Heavily infested, 5 rats fed, 1 lost from cage; (l, m) same as No. 65.

[a] 25 minutes.

[b] 30 minutes.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
68	6 hogs...	100 pounds..	− 9 to 0	10	285	1	1,118	99	1	3	28	1	(f) One out of 2 sluggish; (i) 3 trichinæ; (i, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
69	...do.....	½ pound....	− 9 to 0	10	521	0	1,118	99	0	5	28	1	(g,h) Same as No. 68; (l, m) same as No. 65.
70	...do.....	100 pounds..	− 9 to 0	14	1,442	1	436	99+	0	4	28	1	(f) Very sluggish; (k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
71	...do.....	½ pound....	10 to 12	17	348	54	155	99	1	2	28	1	(f) Less active than normal, 79 out of 188 very sluggish; (i, k) 5 rats fed, 2 died, eaten by others; (i) 4 trichinæ; (l, m) same as No. 65.
72	1 hog.....	.....do.....	− 9 to 0	15	83	0	100	99	0	5	0	4	(l, m) Meat not fed until nearly 8 months after slaughter.
73	...do.....	.....do.....	10 to 12	15	117	76	100	99	1	4	0	4	(f) Less active than normal, 38 out of 89 very sluggish; (i) identity questionable, numerous well-encysted trichinæ 25 days after first feeding; (g, h, l, m) same as No. 72.
74	...do.....	100 pounds..	− 9 to + 2	18	600	0	198	100	0	4	0	4	(k) Five rats fed, 1 died, eaten by others; (l, m) same as No. 72
75	...do.....	½ pound....	− 9 to + 2	20	386	0	198	100	0	4	0	4	(g, h) Same as No. 74; (l, m) same as No. 72.
76	...do.....	.....do.....	10 to 13	20	669	48	198	100	0	4	0	4	(f) Mostly less active than normal, 219 out of 319 sluggish; (g, h) same as No. 74; (l, m) same as No. 72.
77	...do.....	100 pounds..	− 2 to + 5	15	153	0	193	99+	0	5	.....	.....	
78	...do.....	1 pound.....	12 to 16	17	139	50	193	99+	0	5	.....	.....	(f) Mostly quite lively; (g, h) same as No. 77
79	...do.....	380 pounds..	− 2 to + 5	23	90	0	138	100	0	5	.....	.....	
80	...do.....	100 pounds..	− 2 to + 2	15	122	0	138	100	0	5	.....	.....	(g, h) Same as No. 79.
81	...do.....	1 pound.....	13 to 15	17	304	65	138	100	0	5	.....	.....	(f) Mostly quite lively; (g, h) same as No. 79.
82	...do.....	100 pounds..	− 2 to + 2	15	152	0	138	100	0	5	.....	.....	(g, h) Same as No. 79.
83	...do.....	1 pound.....	13 to 15	17	223	42	138	100	0	5	.....	.....	(f) Mostly sluggish; (g, h) same as No. 79.
84	...do.....	100 pounds..	− 2 to + 2	15	50	0	120	100	0	5	.....	.....	
85	...do.....	1 pound.....	13 to 15	18	86	38	120	100	0	5	.....	.....	(f) Mostly sluggish; (g, h) same as No. 84.
86	...do.....	100 pounds..	− 2 to + 2	15	291	0	120	100	1	4	.....	.....	(g, h) Same as No. 84; (i) died 4 days after first feeding, 1 live, 2 dead larvæ in intestine, undeveloped.

87	...do.....	1 pound.....	13 to 15	18	205	67	120	100	0	5	.....	.....	(f) Mostly quite lively, but paler than normal; (g, h) same as No. 84.
88	7 hogs...	.....do.....	4 to 7	14	744	1	143	100	0	5	5	0	(f) Very sluggish; (l) test rats Exp. No. 90, serve as controls for Exp. Nos. 88, 89.
89	...do.....	.....do.....	8 to 11	14	121	98	43	100	2	3	5	0	(f) Not so lively as and paler than normal; (i) slightly infested; (l, m) same as No. 88.
90	...do.....	.....do.....	14 to 16	14	42	100	43	100	5	0	.....	.....	(g, h) Same as No. 89; (i) heavily infested.
91	6 hogs...	½ pound....	5 to 6.5	10	213	0	150	100	0	5	11	1	(l) Eight heavily infested, degree of infestation not recorded in 3; (m) killed 4 days after feeding, only small portion of intestine examined.
92	...do.....	.....do.....	5 to 6.5	11	300	0	150	100	0	5	11	1	(g, h, l, m) Same as No. 91.
93	...do.....	.....do.....	5 to 6.5	13	151	0	100	100	0	5	11	1	(l, m) Same as No. 91.
94	...do.....	.....do.....	5 to 6.5	14	204	2	55	100	0	5	11	1	(f) Very sluggish; (l, m) same as No. 91.
95	...do.....	.....do.....	5 to 6.5	15	202	0	100	100	0	5	11	1	(l, m) Same as No. 91.
96	...do.....	.....do.....	5 to 6.5	16	159	0	153	99	0	5	11	1	(e, g) Digested nearly 2 days; (l, m) same as No. 91.
97	...do.....	.....do.....	5 to 6.5	17	267	0	150	100	0	5	11	1	(l, m) Same as No. 91.
98	...do.....	.....do.....	5 to 6.5	18	139	3	150	100	0	5	11	1	(f) Very sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
99	...do.....	.....do.....	5 to 6.5	20	180	0	100	100	0	5	11	1	(l, m) Same as No. 91.
100	...do.....	.....do.....	5 to 6.5	21	.....	.....	.....	.....	0	5	11	1	Do.
101	...do.....	.....do.....	5 to 6.5	22	340	0	200	100	0	5	11	1	Do.
102	...do.....	.....do.....	5 to 6.5	23	105	0	(?)	100	0	5	11	1	Do.
103	...do.....	.....do.....	10.5 to 13	10	(?)	Many.	150	100	5	0	11	1	(g, h) Same as No. 91; (i) heavily infested, 1 very heavily; (l, m) same as No. 91.
104	...do.....	.....do.....	10.5 to 13	11	(?)	...do.....	150	100	5	0	11	1	(g, h) Same as No. 91; (i) 4 heavily infested, 1 slightly infested; (l, m) same as No. 91.
105	...do.....	.....do.....	10.5 to 13	13	46	33	100	100	5	0	11	1	(f) Very sluggish; (i) 1 heavily infested, others with 1, 22, 35, and 126 trichinæ; (g, h) same as No. 93; (l, m) same as No. 91.
106	...do.....	.....do.....	10.5 to 13	14	50	90	55	100	3	2	11	1	(f) Less active than normal; (i) 20, 7, and 4 trichinæ; (g, h) same as No. 94; (l, m) same as No. 91.
107	...do.....	.....do.....	10.5 to 13	15	36	78	100	100	0	5	11	1	(f) Less active than normal; (g, h) same as No. 95; (l, m) same as No. 91.
108	...do.....	.....do.....	10.5 to 13	16	152	5	153	99	0	5	11	1	(f) Very sluggish, digested nearly 2 days; (g, h) same as No. 96; (l, m) same as No. 91.
109	...do.....	.....do.....	10.5 to 13	17	26	29	150	100	0	4	11	1	(f) Sluggish; (k) 5 rats fed, 1 died, eaten by others; (g, h) same as No. 97; (l, m) same as No. 91.
110	...do.....	.....do.....	10.5 to 13	18	35	31	150	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
111	...do.....	.....do.....	9.5 to 13	20	67	18	100	100	0	5	11	1	(f) Less active than normal; (g, h) same as No. 99; (l, m) same as No. 91.
112	...do.....	.....do.....	9 to 13	21	.....	.....	.....	.....	0	5	11	1	(l, m) Same as No. 91.
113	...do.....	.....do.....	9 to 13	22	(?)	(?)	200	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 101; (l, m) same as No. 91.
114	...do.....	.....do.....	9 to 13	23	74	34	(?)	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 102; (l, m) same as No. 91.
115	...do.....	.....do.....	14.5 to 15	10	(?)	Many.	150	100	5	0	11	1	(f) Commonly less active than normal; (g, h) same as No. 91; (i) 4 heavily, 1 very heavily infested; (l, m) same as No. 91.
116	...do.....	.....do.....	13.5 to 15	11	(?)	...do.....	150	100	5	0	11	1	(f) Commonly less active than normal; (g, h, l, m) same as No. 91. (i) Four heavily, 1 very heavily infested.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls.		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
117	6 hogs...	½ pound....	13.5 to 15	13	(?)	Many.	100	100	5	0	11	1	(f) Commonly less active than normal; (g,h) same as No. 93; (l,m) same as No. 91. (i) Three heavily, 1 very heavily infested, 1 with 43 trichinæ.
118	...do.....	.....do.....	13.5 to 15	14	95	99	55	100	4	1	11	1	(f) Mostly quite lively; (i) 2 heavily infested, 2 with 9 and 3 trichinæ; (g,h) same as No. 94; (l,m) same as No. 91.
119	...do.....	.....do.....	13.5 to 15	15	(?)	Many.	100	100	5	0	11	1	(f) Mostly quite lively; (i) heavily infested; (g,h) same as No. 95; (l,m) same as No. 91.
120	...do.....	.....do.....	13.5 to 15	16	175	26	153	99	5	0	11	1	(f) Very sluggish, digested 2 days; (i) 4 heavily infested, 1 with 4 trichinæ; (g,h) same as No. 96; (l,m) same as No. 91.
121	...do.....	.....do.....	13.5 to 15	17	(?)	Many.	150	100	4	1	11	1	(f) Quite lively; (i) 2 heavily infested, 2 with 5 and 6 trichinæ; (g,h) same as No. 97; (l,m) same as No. 91.
122	...do.....	.....do.....	13.5 to 15	18	(?)	...do.....	150	100	3	1	11	1	(f) Quite lively; (g,h) same as No. 97; (i) heavily infested, 5 rats fed, 1 died, eaten by others; (l,m) same as No. 91.
123	...do.....	.....do.....	13.5 to 15	20	(?)	...do.....	100	100	4	1	11	1	(f) Quite lively; (g,h) same as No. 99; (i) slightly infested, 1, 7, 4, and 18 trichinæ; (l,m) same as No. 91.
124	...do.....	.....do.....	13.5 to 15	21	.....	.....	.....	.....	0	5	11	1	(l,m) Same as No. 91.
125	...do.....	.....do.....	13.5 to 15	22	(?)	(?)	200	100	0	5	11	1	(f) Some quite lively; (g,h) same as No. 101; (l,m) same as No. 91.
126	...do.....	.....do.....	13.5 to 15	23	(?)	(?)	(?)	100	3	2	11	1	(f) Fairly lively; (g,h) same as No. 102; (i) slightly infested, 12, 27, and 1 trichinæ; (l,m) same as No. 91.
127	1 hog....	100 pounds..	10 to 13	57	97	6	52	98	0	5	0	4	(f) Five out of 6 very sluggish, other 1 sluggish; (l,m) same as No. 72.

TABLE II.—*Summary of results of refrigeration experiments with larvæ of Trichinella spiralis exposed to various temperatures*

Exposure to about 15° F.				Exposure to about 10° F.				Exposure to about 5° F.				Exposure to about 0° F.			
Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.
3	2	+	....	50	(a)	+	....	49	(b)	—	....	4	(c)	—	—
7	5	+	....	35	5	+	+	2	(a)	—	—	5	(a)	—	—
17	5	+	—	11	6	+	+	16	5	—	—	6	(a)	—	—
18	5	+	—	36	6	+	—	19	5	—	—	8	2	....	....
20	5	+	—	56	6	+	+	42	5	+	+	9	3	+	—
21a	6	....	—	37	7	+	—	21	6	....	—	10	5	—	—
23	7	+	+	51	7	+	+	43	6	—	—	13	5	—	—
24	9	+	+	57	7	....	+	44	7	+	—	67	5	+	—
25	10	+	—	38	8	—	....	45	8	—	....	1	6	—	+
22	10	+	—	52	8	....	+	46	9	+	—	12	6	+	—
22a	10	....	—	58	8	+	+	47	10	—	—	14	8	—	—
22b	10	....	—	39	9	+	—	91	10	—	—	68	10	+	+
115	10	+	+	53	9	+	+	48	11	—	—	69	10	—	—
26	11	+	+	59	9	....	+	92	11	—	—	70	14	+	—
116	11	+	+	40	10	+	—	93	13	—	—	72	15	—	—
15	12	—	—	54	10	+	+	88	14	+	—	77	15	—	—
27	12	+	—	60	10	....	+	94	14	+	—	80	15	—	—
28	13	+	—	103	10	+	+	95	15	—	—	82	15	—	—
117	13	+	+	41	11	....	—	96	16	—	—	84	15	—	—
29	14	....	+	55	11	+	+	97	17	—	—	86	15	—	—
90	14	+	+	61	11	....	+	98	18	+	—	74	18	—	—
118	14	+	+	104	11	+	+	99	20	—	—	75	20	—	—
119	15	+	+	62	12	+	+	100	21	....	—	79	23	—	—
30	16	+	+	63	13	+	+	101	22	—	—				
120	16	+	+	105	13	+	+	102	23	—	—				
78	17	+	—	64	14	+	—								
81	17	+	—	89	14	+	—								
83	17	+	—	106	14	+	+								
121	17	+	+	73	15	+	—								
31	18	+	+	107	15	+	—								
85	18	+	—	108	16	+	—								
87	18	+	—	71	17	+	+								
122	18	+	+	109	17	+	—								
123	20	+	+	110	18	+	—								
32	21	+	—	65	19	+	+								
124	21	....	—	65a	19	+	+								
33	22	+	—	66	19	+	—								
125	22	+	—	76	20	+	—								
126	23	+	+	111	20	+	—								
34	24	....	—	112	21	....	—								
				113	22	+	—								
				114	23	+	—								
				127	57	+	—								

a 30 minutes. *b* 25 minutes. *c* 10 minutes. *d* 20 minutes.

RESULTS OF EXPERIMENTS

EFFECTS OF VARIOUS LOW TEMPERATURES UPON THE VITALITY OF TRICHINÆ

In only one instance out of 34 experiments in which trichinous meat was exposed to temperatures of about 15° F. for periods ranging from 2 to 23 days were all of the trichinæ upon examination found to be inactive (experiment 15, 12 days). In most instances, although some were found to be inactive, a large proportion were commonly found to be active, not rarely as high as 98 to 100 per cent. In one case, even after 18 days' exposure (experiment 31), over 99 per cent of the trichinæ were found active on examination, and in another case after 22 days (experiment 33) 84 per cent were active.

In 38 experiments test animals were fed meat which had been exposed to about 15° F. for periods ranging from 5 to 24 days, with positive results—i. e., resultant infection—in 17 experiments and negative results in 21.

Some of the negative results were obtained in experiments in which the meat had been kept in the freezer for only 5 and 6 days; on the other hand, positive results were obtained from feeding meat which had been in the freezer for 23 days. Heavy infections were obtained from meat exposed as long as 18 days (experiment 122), but only slight infections resulted from meat kept in the freezer for 20 days or longer (seven experiments), and then only in two instances: In experiment 123 (20 days) one rat was negative, four slightly infested, and in experiment 126 (23 days) two rats were negative, three slightly infested.

From these results it appears that trichinous meat commonly fails to produce infection after exposure to temperatures of about 15° F. for periods of 5 to 24 days, notwithstanding the fact that many trichinæ remain alive and are quite lively when thawed out after such exposure. Failure to infect is probably because, first, of a reduction in the number of live trichinæ and, second, of a reduction in the vitality of those that remain alive. It may be concluded that although a temperature of 15° F. has an injurious action upon the vitality of trichinæ, this temperature is uncertain in its effects and that meat exposed to a temperature of 15° F. for as long as 23 days is still liable to produce infection. These results correspond to those obtained by Schmidt, Ponomarer, and Savelier (1915) who concluded from their experiments that a temperature of −9° C. (+15.8° F.) is sometimes fatal to trichinæ, but not always and that the results of exposure to this temperature are variable and uncertain.

The same authors also found that a temperature of −6° (+21.2° F.) has comparatively little effect upon trichinæ exposed to it for a period of 10 days.

Trichinæ were found to be alive upon examination in 34 out of 35 experiments in which trichinous meat was exposed to temperatures of about 10° F. for periods varying between 30 minutes and 57 days, all but one of the experiments having to do with periods of 5 to 23 days. In the one case in which all of the trichinæ were found to be dead (experiment 38) the meat had been artificially digested for 2 days in preparation for examination instead of less than 24 hours as usual, which is the probable explanation why none was found alive. Although there were no striking differences in the percentages of trichinæ found alive as compared with the findings in the experiments in which meat was exposed to temperatures of about 15°, it was frequently noted that they were less lively than normal, commonly sluggish. In 20 of the experiments a record was made of the degree of activity and it was noted that in 19 of these the trichinæ were sluggish, or at least less lively than

normal, and that in the twentieth they were nearly all very lively (experiment 11, 6 days' exposure). It was quite noticeable in the examinations that the activity of the trichinæ was generally much more impaired than in the case of meat exposed to 15°.

In 41 out of the total of 43 experiments in which meat was exposed to temperatures of about 10° F., test animals were fed, the results being positive in 22 cases, negative in 19. In one of the latter (experiment 73) one out of five rats was found to be heavily infested, but there is a question as to the identity of this rat; furthermore, the trichinæ were too far advanced in development to have resulted from meat fed at the time the rats belonging to this lot were fed. In feedings with meat exposed to temperatures of about 10° for 13 days or less, heavy infestations were commonly produced, but in 17 experiments with meat exposed 14 to 23 days and in one with meat exposed 57 days the results of feeding were either negative or, if infection was produced, it was slight. In only 4 of these 18 experiments did any of the test animals become infested. In experiment 106 (14 days) three rats were slightly infested, two negative; in experiment 71 (17 days) one was very slightly infested (four trichinæ in diaphragm), two negative; in experiment 65 (19 days) four were very slightly infested, one negative; and in experiment 65a (19 days) two were very slightly infested (four trichinæ in the diaphragm of each), two negative.

Summarizing the results of the experiments with meat exposed to temperatures of about 10° F. it may be noted that trichinæ have been found to survive in meat exposed for as long as 57 days, though in that case only a small percentage, and those only sluggishly active, and that some survived in nearly all cases, their numbers and vitality, however, having been so reduced that after 14 days' exposure either no infection resulted in test animals or, if infection resulted, it was very slight. Evidently, therefore, the effects of a temperature of 10° upon the vitality of trichinæ are decidedly more pronounced than those of a temperature of 15°.

Twenty-five experiments were carried out in which trichinous meat was exposed to temperatures of about 5° F.; and in 23 of these, examinations were made of the trichinæ after thawing. In only six instances were live trichinæ found. In experiment 42 (5° to 7° for 5 days) 14 per cent of the trichinæ were found to be alive, degree of activity not recorded. The number of live trichinæ found in the five other experiments ranged from less than 1 per cent to 3 per cent, and they were all very sluggish (experiments 44, 46, 88, 94, 98), the periods of exposure to cold being 7, 9, 14, 14, and 18 days, respectively.

Test animals were fed in 23 experiments. No infections resulted except in experiment 42, just referred to. In this experiment three rats were fed and two became moderately and one slightly infested.

The results of these experiments show that temperatures of about 5° F. have a profound effect upon the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are extremely unlikely to occur, none having resulted in any case in which test animals were fed meat exposed to temperatures of about 5° for periods ranging from 6 to 23 days (19 experiments). In view of the results of experiment 68, however, in which the temperature was −9° to 0° and the period of exposure 10 days, it may be concluded that slight infections may sometimes result from meat exposed to 5° for as long as 10 days.

The results of the experiments with temperatures of about 5° F. correspond closely to those of Schmidt, Ponomarer, and Savelier (1915). These authors, however, found that in their experiments a temperature of −15° to −16° C. (3.2° to 5° F.) was always fatal to trichinæ and noted no exceptions such as were observed by the present writer.

In experiments in which trichinous meat was exposed to temperatures of about 0° F., but ranging as low as −10° in some instances, trichinæ were rarely found to be alive. However, 100 per cent were found to be alive in one experiment (No. 67) in which meat had been exposed to a temperature of −4° to 0° for 5 days, but in 15 experiments in which the period of exposure to cold ranged from 6 to 23 days trichinæ were found alive only in three instances and less than 1 per cent in each case (experiments 12, 68, and 70).

Test animals were fed in all but 1 of the 23 experiments with temperatures of about 0° F. Infection resulted in two instances. Four rats fed in experiment 67 (−4° to 0°, for 5 days) became heavily infested, and one out of four in experiment 68 (−9° to 0°, for 10 days) showed three trichinæ in the diaphragm, the three other rats being negative. In the latter case, as in the former, live trichinæ had been found by examination of the meat; less than 1 per cent, however, as compared with 100 per cent in the former, the results of the feeding tests thus as usual being quite consistent with the results of the examinations of artificially digested meat, though it was unusual for infection to result when the examination showed such a small percentage of live trichinæ as in experiment 68. In experiment 86 (−2° to +2°, for 15 days), in which no trichinæ were found alive on examination of artificially digested meat, the result of the feeding test is considered to have been negative, although one of the five test rats, which died four days after feeding, was found to have three trichina larvæ in the intestine, two of which were dead, whereas the other one exhibited feeble movements. None of these three larvæ, however, had undergone any development, and the four other test rats were negative, so that it seems quite proper to conclude that the viability of the trichinæ had been destroyed in the meat in question.

From the foregoing it appears that the results of exposing trichinous meat to temperatures of about 0° F. are similar to those produced by temperatures of about 5°—i. e., a few trichinæ may survive exposures to such temperatures for 6 days or more, but their vitality will be so greatly reduced that there is little likelihood of their causing infection, although, on the other hand, slight infections may result from meat exposed as long as 10 days.

A good example of the relative effects of different low temperatures upon the vitality of trichinæ is supplied by experiments 91 to 126. In these experiments approximately equal quantities of trichinous pork from the same source (mixture of meat from six hogs) were exposed for 10 to 23 days in three freezers at temperatures of about 15°, 10°, and 5° F., respectively, a can of meat being removed from each of the three freezers after 10 days' exposure, another after 11 days, and so on (no cans, however, being removed on the twelfth or nineteenth day). It will be observed from the recorded results (Tables I, II) that many of the trichinæ in the meat exposed to a temperature of about 15° survived, and up to the twentieth day of exposure were mostly quite lively after thawing. Some of those from meat exposed for 22 days were observed to be quite lively, and those which survived in meat exposed for 23 days were found to be fairly lively. From the results of the feeding tests there appeared to be a considerable reduction in the vitality of the parasites after 17 days' exposure, notwithstanding the survival of a large percentage. Most of the rats fed meat exposed to about 15° for 10 to 16 days became heavily infested, but the 17-day meat failed to infect one out of five rats, and only two of the four others became heavily infested, the 18-day meat failed to infect one out of five, the 20-day meat failed to infect one, the four others becoming only slightly infested, none of the rats fed 21- and 22-day meat became infested, and the 23-day meat failed to infect two and produced only light infestations in the three others.

In the case of the meat exposed to a temperature of about 10° F. it was observed that the trichinæ which survived were relatively less numerous, as a rule, than in the case of the meat exposed to about 15°, and it was generally noted that they were less active than normal, or sluggish, sometimes very sluggish. The test rats, fed meat exposed for 10 days, all became heavily infested, all five fed 11-day meat became infested, but one was only slightly infested, all five fed 13-day meat became infested, but only one was heavily infested, three out of five fed 14-day meat became infested, but these only slightly, and none of the rats fed meat exposed to about 10° for 15 days or longer became infested. In this series, therefore, there was apparently a considerable reduction in the infectiousness of the meat beginning with that exposed for 13 days, and after 2 days more the infectiousness became nil.

Practically none of the trichinæ in the meat exposed to a temperature of about 5° F. (experiments 91 to 102) survived; although living trichinæ

were observed in meat exposed for 14 and 18 days (2 and 3 per cent, respectively), these were very sluggish. Furthermore, none of the test rats in this series became infested.

The results of the three sets of experiments just cited demonstrate quite clearly that a temperature of 10° F. is more effective in destroying the vitality of trichinæ than a temperature of 15°, and that a temperature of 5° is still more effective, illustrating the general rule established by the investigations recorded in the present paper, that within certain limits the effect upon the vitality of trichinæ becomes more pronounced as the temperature of refrigeration is lowered. It has also apparently been established that the increase in effectiveness is not uniform with the decrease in the temperature, but that somewhere in the neighborhood of 10° a critical point is reached, below which there is a sudden increase in the effectiveness of refrigeration.

Summarizing the results of the various experiments with a view to their practical application, inasmuch as very few trichinæ have been found to survive an exposure of more than 10 days to a temperature of 5° F., or lower, and as the few surviving have shown only very slight activity, and as, moreover, trichinous meat exposed to temperatures of 5° or lower has rarely produced infestation, and has never (in repeated trials) produced infestation when the period of exposure was more than 10 days, it may be concluded that meat exposed to a temperature not higher than 5° for a period of 20 days will no longer contain viable trichinæ, 10 days in this 20-day period being allowed as a margin of safety. It may be further concluded that, so far as our present knowledge goes, temperatures of 10° and higher are too uncertain in their effects upon the vitality of trichinæ to justify the use of refrigeration at such temperatures as a means of rendering trichinous meat innocuous.

CHANGES PRODUCED IN TRICHINA LARVÆ BY EXPOSURE TO LOW TEMPERATURES

Low temperatures (15° F. and lower) not only destroy the vitality of some or all of the trichinæ which are exposed to those temperatures but they produce changes in the tissues of the parasites, which are apparent under the microscope. These changes in appearance are associated with reductions in the activity of the trichinæ and with losses in their vitality.

Trichinæ from artificially digested unfrozen meat when examined under the microscope in water, or preferably in a physiological salt solution are found to be tightly coiled, becoming very lively when they are warmed to body temperature and continuing their lively movements as the temperature increases up to about 50° or 52° C. when they become sluggish and finally cease movement and die when the temperature rises a few degrees higher. The esophageal cellular body of the normal trichina has a bright yellowish brown color, and exhibits a certain granulation of the protoplasm; the nuclei of the cells are apparent as small,

clear, spherical bodies, seemingly of a vesicular nature. The gonad (ovary or testis) forms a continuous mass of cells closely pressed together, intercellular divisions and nuclei being indistinct in the living specimen. The body cavity forms a thin but distinct space between the internal organs and the parietal wall. In short, the normal living trichina larva freed from its capsule by artificial digestion presents a sharp clear-cut bright appearance which is quite characteristic but difficult to describe.

The changes shown by the trichinæ from artificially digested meat in experiments 118, 106, and 94 are typical of those produced by the exposure of trichinous meat to various low temperatures. In these instances the temperatures were 13.5° to 15°, 10.5° to 13°, and 5° to 6.5° F., respectively, and the period of exposure 14 days in each case. The meat was all of the same origin—i. e., from six hogs, mixed together, portions of about half a pound being inclosed in tin cans and placed in freezers maintained at the temperatures stated. The cans were removed at the end of 14 days and the meat allowed to thaw at ordinary temperatures. Two days after removal from the freezers the meat from each can was ground up, digested overnight in an artificial gastric juice, washed and sedimented in a 0.6 per cent salt solution and the trichinæ thus obtained subjected to examination. As usual, for the purpose of controlling the results of these processes upon the frozen meat, unfrozen meat from the same carcasses was digested, washed, and examined in exactly the same manner.

Out of 95 trichinæ from the meat which had been exposed to a temperature of 13.5° to 15° F. (No. 118), only one was inactive, this one being pale in color, and the nuclei in the cellular body having a solidified appearance. The 94 others were more or less tightly coiled when cold, and most of them were quite lively when warmed. The granulation of the protoplasm of the cellular body differed only slightly from normal, and its color was nearly normal; the nuclei showed commonly a small central point of more solid appearance than the remainder of the nucleus. The gonad either showed only slight changes from normal or the germ cells were rounded instead of being closely pressed together, this rounding of the cells occurring in only a part of or throughout the gonad. Two of the test rats in this experiment became heavily infested; one was negative; one showed 9 trichinæ in the diaphragm; and one 3 trichinæ in the diaphragm.

Fifty trichinæ were examined from the meat which had been exposed to a temperature of 10.5° to 13° F. Of these, five were inactive, pale in color, their coils expanded so that they resembled a figure 6, and the nuclei of the cellular body of the esophagus were solidified. The 45 which were active were more or less tightly coiled when cold, some of them being quite lively when warmed. The color of the cellular body was rather paler than normal, the protoplasm abnormally granular, the nuclei either not apparent or exhibiting a solidified central portion. The cells of the

gonad were rounded instead of being closely pressed together as in the normal trichina. Two out of five test rats were negative, the three others contained 4, 7, and 20 trichinæ, respectively, in the diaphragm.

In experiment 94, in which the meat had been exposed to a temperature of 5° to 6.5° F., 204 trichinæ were examined, 199 of which were inactive, and only 5 of which showed any activity when warmed, this consisting of a very slight movement on stimulation with a needle point. The coils were expanded in the form of a figure 6, or in some instances formed a very loose spiral. The esophageal cellular body was very pale in color, granulation of the protoplasm very abnormal, nuclei solidified, quite different in appearance from the normal vesicular nucleus. The cells of the gonad were rounded and more or less dissociated. Five test rats fed in this experiment all failed to become infested.

The abnormal granulation of the cellular body referred to is difficult to describe, but it gives the protoplasm a distinctly different appearance from that of the cellular body of an unfrozen trichina, dull and dead-looking as compared with the bright appearance of the latter, the visible particles being much more numerous and smaller.

Comparison of the results of these three experiments and similar experiments shows not only that microscopically visible changes occur in the minute structure of trichinæ subjected to temperatures of 15° F. and lower, but that these changes are more pronounced in trichinæ subjected to about 10° than in those subjected to about 15°, and still more pronounced in trichinæ subjected to about 5°. These changes are evidently brought about by the low temperature, but in what way is not apparent. This problem probably belongs in the field of colloid chemistry. There occurs perhaps a precipitation of the colloids in the tissues of the trichina or some change in their nature which is more or less irreversible, according as the temperature is lower or higher and the period of exposure longer or shorter. In those cases in which the trichinæ were examined very soon after thawing of the meat (experiments 1 and 3, for example) it was quite evident from the shriveled appearance of the parasites that fluid had been extracted from them during their exposure to cold. Trichinæ thus shriveled absorb moisture after thawing and soon lose their shriveled appearance, again becoming active unless the temperature was too low and the period of exposure to cold too long continued. In some respects trichinæ which have been frozen at a low temperature (5° F.) resemble those which have been dried and then moistened again. Ordinary drying, however, destroys the vitality of trichinæ immediately, and the changes produced are much more marked than those produced by freezing. It is possible that the latter might be more closely simulated if the trichinæ were very gradually dried and the drying process stopped at the proper point. As yet, however, careful experiments along this line have not been carried out.

In view of the recent discovery by plant physiologists (see Bachmann, 1914) that sugar in plant tissues acts in some manner to protect them from the injurious effects of freezing so that the same species of plant is able to withstand a lower temperature when its tissues are loaded with sugar than when they contain only small quantities of this substance, it is of interest to note that larval trichinæ contain a high percentage of glycogen.

Whatever may be the explanation of the destruction of the vitality of trichinæ and of the changes brought about by exposure to cold, the investigations thus far carried out are sufficient to prove that trichinæ when exposed to temperatures of 15° F. or lower undergo changes in their protoplasmic structure, and if the temperature is low enough and the exposure to cold continued long enough these changes become so pronounced and so well established that the vitality of all of the parasites is entirely destroyed.

VARIATIONS IN VITALITY OF TRICHINÆ

It is natural to expect that individual trichinæ would vary in resistance to the effects of cold, and this was found to be the case. Some succumb much more quickly and at higher temperatures than others. In order to avoid misleading results on this account, meat was not used in the experiments unless heavily infested so that large numbers of trichinæ might be available for study, considerable quantities were used, as a rule, for examination and for feeding tests, several test animals (four to six) being generally employed; and, commonly, mixed meat from several hogs was used so that the chances of including only feebly resistant trichinæ in an experiment may be considered to have been reduced to a minimum in most cases.

QUANTITIES OF MEAT FROZEN

As already noted, various quantities of meat ranging from a gram or two up to nearly 400 pounds in weight were frozen in the various experiments. The rate of freezing and thawing, of course, varied with the quantity of meat, the change of temperature being rapid when small quantities, slow when large quantities were used. When very small quantities of meat or of fluid containing free trichinæ were frozen and thawed within a few minutes (experiments 2, 4, 5, 6, 49, 50) the trichinæ were apparently much more injuriously affected than when larger quantities of meat were subjected to similar temperatures for considerably longer periods of time. On the other hand, if the quantity of meat weighed half a pound or more, differences in the weight, and consequently in the rate of freezing and thawing, made no appreciable difference in the effect upon the vitality of the trichinæ, as is quite evident from a comparison of the various experiments recorded in the tables. In short, it may be

stated that if the temperature to which trichinous meat is exposed is sufficiently low and the length of exposure sufficiently long, the trichinæ are killed just as certainly when large quantities of meat are frozen as when small quantities (not less than half a pound) are frozen, variations in the rate of freezing and thawing dependent upon variations in the quantity of meat frozen being immaterial.

VARIATIONS IN LENGTH OF TIME AFTER REMOVAL FROM FREEZER BEFORE EXAMINING AND TESTING MEATS

In some cases examination of the trichinæ from meat which had been frozen was made on the same day the meat was removed from the freezer or freezing mixture. When the meat was digested before examination, it was in some instances placed in the digesting fluid the same day the meat was removed from the freezer, but generally one or more days up to a maximum of 12 days elapsed before the meat was digested and examined, and a corresponding period before the feeding of test animals was begun.

Nearly all of the experiments were carried out in cold weather, and the meat after thawing, except when in transit to the laboratory, was kept in coolers or ice boxes until it was placed in a digesting fluid or fed to test animals, so that decomposition changes were slight.

In the majority of instances the meat was placed in digesting fluid in preparation for examination and the feeding of rats begun within four days after removal from the freezer, but longer periods appeared to have no pronounced effect upon the results. Certainly the lapse of time did not favor the revival of the trichinæ. For example, in experiments 77, 80, 82, 84, and 86 the periods which elapsed between removal from the freezer (about 0° for 15 days) and the digestion of the meat were 12, 8, 8, 10, and 10 days, respectively; and between removal from the freezer and the first feedings of test animals, 13, 8, 8, 10, and 10 days, respectively, yet no trichinæ were found alive on examination, and none of the test animals became infested. On the other hand, it did not seem that the lapse of time following removal from the freezer had much effect in reducing the vitality of surviving trichinæ, though it is quite likely that the longer the period which elapses after trichinous meat is removed from the freezer the fewer the surviving trichinæ will be, other things being equal. In experiments 126, 81, 83, 85, 87, and 78, the periods elapsing between removal from the freezer (about 15°, 17 to 23 days) and digestion of the meat were 6, 6, 6, 7, 7, and 9 days, respectively, and between removal from the freezer and the first feedings of test animals 4, 6, 6, 1, 7, and 10 days, respectively. A high percentage of trichinæ were found to be alive in each case. In only one of the experiments in question (No. 126) did any of the test animals become infested, and this might be taken to indicate that the trichinæ had suffered somewhat

because of the longer periods elapsing since the removal of the meat from the freezer, inasmuch as in other experiments in which the period of exposure in the freezer had been about the same but in which the meat was fed more promptly positive results were obtained in the feeding tests—i. e., in experiments 31, 122, 123, and 121, the periods elapsing between removal from the freezer and the first feeding of test animals being 2, 2, 2, and 3 days, respectively. This comparison, however, is not of great value, since in experiments 15, 28, 27 (meat in freezer at about 15° F. for 12 to 13 days), and 125 (meat in freezer at about 15° for 22 days) in which the meat was fed 2, 5, 5, and 4 days, respectively, after removal from the freezer, the results of the feeding tests were negative.

Further investigation is required to determine the changes which occur in the vitality of trichinæ when frozen meat is kept for varying periods of time after thawing. From the data at present available, however, it is quite certain that if any considerable changes occur, they are in the direction of a lowering of vitality and not in the reverse direction.

In this connection it is of interest to note that in unfrozen meat kept over three months after slaughter the trichinæ had suffered no evident loss in vitality, and small quantities of the meat were sufficient to produce heavy infestations in rats (controls, experiments 91 to 126). On the other hand, in meat kept nearly eight months after slaughter the trichinæ had lost their vitality, and test rats failed to become infested (controls, experiments 72 to 76).

EFFECTS OF ARTIFICIAL DIGESTION UPON TRICHINÆ

As evident from the tabular statement of the experiments (control examinations), artificial digestion for 24 hours or less had no appreciably injurious effect upon the vitality of trichinæ. When digested for two days, however, a considerable proportion of the trichinæ are liable to be killed (experiment 32). On the other hand, if 5 or 6 gm. of salt are added to each liter of digestive fluid the vitality of the trichinæ is not so seriously affected. The trichinæ from unfrozen meat digested for two days in experiment 96 seemed as lively as usual. Trichinæ, however, from meat frozen for 16 days at about 15° F. in experiment 120 evidently suffered considerably from digestion for two days, inasmuch as a smaller proportion were active and these were less lively than trichinæ examined in experiments 121, 122, 123, from meat frozen 17, 18, and 20 days, respectively, at about 15° F. and digested less than 24 hours. Furthermore, the fact that prolonged digestion in a digestive fluid containing 0.5 per cent of sodium chlorid is injurious to trichinæ from unfrozen meat was shown by an experiment in which digestion was continued for four days. In this instance all of the trichinæ were killed.

Though it is possible that the methods of artificial digestion employed in the experiments to free trichinæ from meat for examination reduced

their vitality so that many were found to be inactive which before digestion were still alive, the results of the examinations corresponded very well with the feeding tests. In fact, the examinations not uncommonly showed some of the trichinæ to be still alive, whereas in the corresponding feeding tests with the same meat not artificially digested none of the test animals became infested. On the other hand, there was no case in the freezing experiments in which the feeding test resulted in infection and the corresponding examination failed to reveal living trichinæ unless experiment 86 be taken as an exception. In this experiment, following a negative examination of digested meat, 3 larval trichinæ were found in the intestine of one of the test rats, which died four days after the first feeding; one of these larvæ was alive and exhibited feeble movements, but none of the 3 had undergone any development; the 4 other test rats failed to become infested. Experiment 67 was nearly an exception to the rule, as only 2 live trichinæ were found among 285 examined, the feeding test resulting positively. Only one out of four test rats became infested, however, and this one had but 3 trichinæ in the diaphragm. On the whole, the method of artificial digestion appears to afford a more rigorous test of the viability of trichinæ than the feeding of experimental animals in view of the fact that trichinæ are often found to be alive in digested meat when the feeding of the undigested meat to experimental animals fails to produce infection.

As a rule, in testing meat it is preferable not to depend alone upon the results of artificial digestion or the results of feeding test animals, but to employ both methods and take the results of both into consideration.

It is quite evident from the results of the experiments that artificial digestion is a valuable method for testing the viability of trichinæ, and that when properly controlled its injurious effects upon their vitality are so slight as to be practically negligible. The following formula may be recommended as fully satisfactory:

Water	1,000 c. c.
Hydrochloric acid (sp. gr. 1.19)	10 c. c.
Scale pepsin (U. S. P.)	2.5 gm.
Sodium chlorid	5 gm.

Fifty grams of ground meat are to be stirred into 600 c. c. of the digesting fluid, warmed to 38° or 40° C., and incubated for about 18 hours at this temperature.

LONGEVITY OF TRICHINÆ AFTER ARTIFICIAL DIGESTION

Trichinæ freed from their capsules by artificial digestion have been kept alive in tap water for 15 days. In one case 73 out of 75 were active at the end of this time. When examined again, 13 days later, all were dead. Kept in a 0.6 per cent sodium-chlorid solution for 16 days, 41 out of 43 examined were alive, some of them being sluggish but most of them

moderately lively. In another lot kept in a 0.6 per cent sodium-chlorid solution for 26 days, 15 out of 24 were alive and moderately active when warmed. Examined again 24 days later, all were dead. In a lot kept in 2 per cent sodium-chlorid solution for 11 days, 37 out of 38 were alive and very active. In these instances, after digestion of the meat, the trichinæ were washed in several changes of water or in physiological salt solution by decanting and settling. They were kept at ordinary room temperature. Numerous observations were made which showed that trichinæ freed from their capsules by artificial digestion will be apparently just as lively after several days if kept in water or physiological salt solution at ordinary room temperature as they are immediately after digestion.

If tap water containing trichinæ is kept at a temperature of 38° C. most of them are killed in a short time, but trichinæ may be kept an equal length of time at this temperature in a 0.6 per cent sodium-chlorid solution without apparent injury as shown by the following: Trichinæ from artificially digested meat were separated into two lots in beakers, one containing tap water, the other a 0.6 per cent sodium-chlorid solution. The two beakers were heated to 38° C. and this temperature maintained for 2½ hours. At the end of this time 23 out of 32 trichinæ from the tap water were inactive, whereas 18 examined from the salt solution were all active. The two beakers after replacing the tap water in one with a 0.6 per cent sodium-chlorid solution were kept at room temperature until the following day and then reexamined. Out of 108 trichinæ examined in the one case (heated in tap water), 81 were found to be inactive, whereas in the other case (heated in salt solution) all but 1 out of 100 examined were active.

On another occasion some trichinæ in tap water were kept at a temperature of 32° C. for about half an hour. Most of them became inactive but resumed their activity when the water was replaced with a 0.6 per cent sodium-chlorid solution, although their color became darker than normal and vacuoles appeared in the lateral fields.

It was on account of this discovery of the injurious effects of warm tap water that in the later experiments when meat was digested artificially it was washed with salt solution instead of tap water, and that salt solution instead of tap water was used as an examination medium. The use of tap water in the earlier experiments, however, probably affected the results of the examinations little, if any, as they are evidently quite consistent with the results of the later experiments (see Tables I and II). The washing was done with cold tap water, and in examining the trichinæ they were transferred a few at a time to a warm stage, where they were kept only a few minutes, too short a period for the injurious effects of immersion in warm water to become established, as was repeatedly demonstrated in using this method upon trichinæ from unfrozen meat.

TEST ANIMALS

It will be noted from Table I that of the 54 test animals (53 rats, 1 guinea pig) fed with unfrozen meat as controls upon the animals fed with frozen meat, only 3 failed to become infested. The rats fed as controls in experiments 72 to 76 are left out of consideration, as they were not fed until nearly eight months after the slaughter of the hog from which the meat was taken. Examination of some of the meat artificially digested nine months after slaughter of the hog showed that the trichinæ were dead. One out of three rats fed as controls in experiments 23 to 34 showed no infection, the two others being heavily infested. Out of 29 rats fed as controls in experiments 65, 65a, and 67 to 71, 1 showed no infection, 27 of the remaining 28 showing heavy infections. Finally, 1 out of 12 rats fed as controls in experiments 91 to 126 showed no infection, but this one was killed four days after feeding for another purpose and as only a small portion of the intestine was examined, trichinæ may have been present and were not discovered; 8 of the remaining rats were heavily infested; in the case of the 3 others the degree of infestation was not recorded.

These results, particularly in view of the fact that the control animals as a rule received much smaller quantities of meat than those fed on meat which had been frozen, demonstrate the adequacy of the methods employed in feeding test animals. The results of the later experiments, however, beginning with No. 23 are considered more reliable, so far as the feeding tests are concerned, than those of the earlier experiments, as more animals were used and care was taken to feed larger quantities of meat. The method of feeding each lot of test rats together in a cage a certain amount of meat on several successive days, followed in most of the experiments, appeared to be quite satisfactory. Undoubtedly some of the rats in each lot ate more of the meat than others, so that some inequality in the degree of infestation of the rats would be likely, which, however, was of little importance, as the results of the feeding tests were judged upon the basis of the findings in all of the rats in each lot. The use of a number of rats for each test allowed larger quantities of meat to be tested, which gives a decided advantage over the use of a single animal. For the same reason, rats are preferable to guinea pigs, as they will eat of their own accord much larger quantities of meat than can readily be fed to guinea pigs forcibly or by mixing with lettuce, cabbage, etc. Furthermore, it is difficult to induce guinea pigs to eat chopped meat mixed with lettuce or other materials if the meat has become only slightly tainted, whereas rats usually eat meat readily even after it has become very stale or partially decomposed.

SUMMARY AND CONCLUSIONS

Prior to the investigations recorded in the present paper very little experimental work had been done upon the effects of cold upon encysted trichinæ, and the current belief was that low temperatures do not seriously affect the vitality of these parasites. This belief is shown to have been erroneous by the results of numerous experiments.

Quantities of trichinous meat varying in weight from a few grams to nearly 400 pounds were frozen and kept for periods varying from a few minutes to 57 days at various temperatures below the freezing point of water. Usually the process of refrigeration was carried out in cold-storage compartments known as freezers, but in a few cases in which the low temperature was maintained only a short time, a freezing mixture was employed. In most cases the period of refrigeration was between 5 and 20 days. The meat on removal from the freezer was generally allowed to thaw slowly at ordinary house temperatures; in a few cases, in order to study the effects of rapid thawing, the process was hastened by breaking apart the pieces of frozen meat so that they thawed completely in a few minutes. Generally the meat after thawing was treated as follows: A portion was chopped or ground into fine pieces, placed in an artificial gastric juice, and incubated at 38° to 40° C. overnight, and then washed with water or a physiological salt solution by decanting and sedimenting. The sediment containing the trichinæ isolated from their capsules was examined microscopically on a warm stage, and the number of inactive and active ones recorded, together with such other observations as appeared worthy of remark. For the purpose of controlling the effects of the process of digestion, some trichinous meat, nearly always from the same carcass as the frozen meat, which had been kept in an unfrozen condition, was digested at the same time, using some of the same lot of digesting fluid. Another portion of the frozen meat after thawing was fed to test animals, in most cases to white or hooded rats specially reared to avoid chances of accidental infection; as a rule, five rats were fed, receiving the meat on several successive days. Finally, unfrozen meat from the same carcass as that used in a given refrigeration experiment was fed to control test animals, usually in much smaller quantities than in the case of the frozen meat. In some instances no control test animals were fed. The test animals as they died, or after about a month if they lived that long, were examined for trichinæ, the intestines as well as the diaphragm being examined if they died within the first two weeks after feeding; otherwise only the diaphragm. About 30,000 trichinæ were examined from artificially digested frozen and unfrozen meat, and over 500 test animals and control animals were fed and examined.

A considerable proportion of the trichinæ in meat exposed to a temperature of about 15° F. for periods of 23 days or less survive and are

quite lively after thawing, but such meat frequently fails to infect test animals. This temperature is injurious to trichinæ, but its effects are uncertain, and meat exposed as long as 23 days has proved to be infectious. Some of the trichinæ in meat exposed to a temperature of about 10° for periods of 57 days or less generally survive, but the meat frequently fails to infect test animals. A temperature of 10° is more injurious to trichinæ than a temperature of 15°, but, like the latter, its effects are uncertain, although meat exposed to it for 14 days or longer has generally failed to produce infestation; or if infestation resulted it was slight. No infestation has been produced by trichinous meat exposed to a temperature of about 10° for 20 days or longer.

Apparently in the neighborhood of 10° F. a critical point is reached below which the effects of cold upon trichinæ become suddenly much more pronounced.

Temperatures of 5° F. or lower profoundly affect the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are very unlikely to result. Slight infections, however, have resulted from meat exposed to a temperature of −9° to 0° for 10 days.

Trichinæ from meat exposed to temperatures below 15° F. when examined microscopically after thawing exhibit changes in the appearance of the protoplasm. A temperature of 10° produces greater changes than 15°, and the changes produced by a temperature of 5° are still more pronounced. The more conspicuous of these changes are more or less loss of color of the esophageal cell body, more or less solidification of its nuclei, abnormal granulation of its protoplasm, and more or less dissociation and rounding of the germ cells.

Trichinæ vary in their resistance to cold, and some individuals survive refrigeration longer and at lower temperatures than others.

Within certain limits the rapidity with which trichinous meat freezes or thaws has no appreciable effect upon trichinæ. Apparently the rapid freezing and thawing undergone by very small pieces of meat (a few grams in weight) adds to the injurious effects of refrigeration, but the natural variations in the rate of freezing and thawing dependent upon variations in the quantities of meat frozen between limits of half a pound and several hundred pounds do not noticeably modify the effects of refrigeration upon trichinæ.

The vitality of trichinæ which survive refrigeration does not decrease noticeably during a period of at least a week after the thawing of the meat.

The artificial digestion of trichinous meat for 24 hours at a temperature of 38° to 40° C. in a fluid consisting of water, 1,000 c. c., hydrochloric acid (sp. gr. 1.19), 10 c. c., scale pepsin (U. S. P.), 2.5 gm., and sodium chlorid, 5 gm., has no apparent effect upon the activity or structure of the trichinæ, released from their capsules by the process of digestion.

Trichinæ thus released from their capsules may remain alive and retain their normal activity for 10 days or more when kept in a 0.6 per cent sodium-chlorid solution at a temperature of about 20° C., and have been found alive and moderately active at the end of 26 days. They may likewise be kept alive for two weeks or more in tap water at a temperature of about 20° C. Trichinæ have been kept alive and very active for 11 days in a 2 per cent sodium-chlorid solution at a temperature of about 20° C. Trichinæ in tap water warmed to a temperature of 38° C. become inactive within a few hours, but may be kept in a 0.6 per cent sodium-chlorid solution at this temperature for a similar length of time without apparent injury.

In the practical application of refrigeration as a means of destroying the vitality of trichinæ, meat should be refrigerated at a temperature not higher than 5° F. for not less than 20 days, a period which allows a probable margin of safety of nearly 10 days. The employment of higher temperatures of refrigeration as a means of destroying the vitality of trichinæ is not justified in the light of our present knowledge because of the uncertainty of the effects of such temperatures. Whether temperatures higher than 5° F. may be safely employed by lengthening the period of refrigeration remains to be determined.

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RELATION BETWEEN CERTAIN BACTERIAL ACTIVITIES IN SOILS AND THEIR CROP-PRODUCING POWER

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INTRODUCTION

Soil-bacteriological investigations in the past have dealt almost exclusively with the occurrence and activities of micro-organisms in the soil, and no attempt has been made, from the standpoint of crop production, to interpret the results obtained.

A knowledge of the relation of soil bacteria to soil fertility is of considerable importance, however, if the subject is to be of any value in practical agriculture. While, therefore, much work on methods remains to be done, so much knowledge concerning bacterial action in soils has been accumulated during the last few years that it seems time now to call attention to the practical phase of the subject, to attempt at least to correlate the results secured with known facts regarding soil fertility.

The purpose of these experiments has been to study certain bacterial activities in field soils in the attempt to secure information regarding their relation to the actual crops produced. If special methods of soil treatment exert similar effects on certain bacterial activities and on crops, it may be assumed that there is a fairly definite relation between the two, and the particular bacterial activities in a soil may indicate its crop-producing power. Thus, if in laboratory tests the ammonifying power, the nitrifying power, or the azofying power of a soil is enhanced by some method of soil treatment and the crop production is also increased, the conclusion that ammonification, nitrification, or azofication and crop production are very closely related would be well warranted. Tests of such bacterial action in soils would therefore constitute a means of ascertaining their crop-producing power, and the importance of obtaining advance information along this line is evident.

Experiments covering many years of varying seasons and including tests of all varieties of treatments must, of course, be carried out before any definite conclusions can be reached. The experiments reported here were secured on three series of plots under definite systems of treatment, and it was intended in undertaking the work to carry it on for a long period of years before attempting to draw conclusions. Inasmuch, however, as the particular plots were of necessity relinquished, owing to the development of certain departments of the State College, and studies of a like nature can not be undertaken on new plots until several years of special treatment have elapsed, it has been deemed

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bv

Vol. V, No. 18
Jan. 31, 1916
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expedient to assemble the data obtained up to the present time and to offer them as a preliminary contribution along this line. The fact that many of the data are rather positive in nature has been an added reason for presenting them at this time. Portions of the results have been published in other connections, while others have not previously been reported, but in either case average results only are included here.

FIELD SOILS STUDIED

Three series of field plots have been used in this work, one consisting of 14 plots one-tenth of an acre in size, located on a uniform soil in the Wisconsin drift-soil area, and classed by the United States Bureau of Soils as Carrington loam.

Prior to 1907 it had been under a regular 4-year rotation and had been subjected to no special treatment of any kind. In that year the plots were differentiated according to the following plan:

Plot No.	Treatment.
601	Continuous corn.
602, 603	2-year rotation: Corn and oats.
604, 605, 606	3-year rotation: Corn, oats, and clover.
607, 608	2-year rotation: Corn and oats, clover plowed under after the oats.
609, 610	2-year rotation: Corn and oats, cowpeas plowed under after the oats.
901, 902	2-year rotation: Corn and oats, rye plowed under after the oats.
903	Continuous clover.
904	4-year rotation: Corn, oats, and clover.

The first tests of these soils were carried out in 1911, the fourth year of the special treatment. Results were secured also in 1912 and 1913, only a few data being obtained in the latter year owing to the pressure of other work, but the ammonification studies were complete. During each season only those plots under corn were examined, as the effects of previous treatment could, of course, hardly be studied on plots under different crops, and furthermore it would be evidently impossible to compare the crop yields on the various plots if the same crop were not grown. Different plots in this series were thus examined in the different years, but in each case the same treatments were included in the study.

The second series of plots consisted of 5 one-tenth-acre plots on the same soil area and on the same soil types as the previous series. In the fall of 1910 these plots were subjected to the special treatments indicated below:

Plot No.	Treatment.
1004	Check.
1005	8 tons of manure per acre.
1006	12 tons of manure per acre.
1007	16 tons of manure per acre.
1008	20 tons of manure per acre.

The study of these plots was carried out in 1912, the crop grown that year being corn.

The third series of plots was composed of 3 one-twentieth-acre plots located on the same soil type as the other series.

Special treatment on these soils consisted in the application of lime as follows:

Plot No.	Treatment.
510	Check.
509	2 tons of ground limestone per acre.
508	3 tons of ground limestone per acre.

The lime was applied to these plots just prior to the corn planting, and the tests of the soils were carried out later in the same season.

BACTERIOLOGICAL METHODS

The solution method for testing bacterial activities in soils has been studied in some detail by several investigators, and, while results of much value have been secured by its use, there are certain difficulties attendant upon it which have not yet been obviated. These difficulties have been discussed in another publication [1] and need not be entered upon here. The use of soil itself as a medium for studying bacterial activities in field soils seems at the present time the most logical method. Modified solutions such as have been suggested in recent work [2] can hardly be considered as satisfactory as soil itself in representing the physical and chemical conditions in field soils, leaving out of account entirely the bacteriological factor.

The addition of various materials to soils in laboratory tests to permit the accumulation of the particular products of bacterial action which it is desired to measure has been studied. Dried blood, cottonseed meal, and casein have proved the best for ammonification; dried blood and ammonium sulphate for nitrification; and mannite for azofication.

In this work various modifications of the soil method were employed for the reason that the tests were carried out during a period of several years through which experiments on methods were also being conducted. The results, using the different methods, are all included, however, as they all tend in the same direction, and conclusions are based on a study of the entire mass of data secured.

EXPERIMENTAL WORK

TESTS ON ROTATION PLOTS IN 1911

Four samplings were made during 1911—on June 26, July 8, September 16, and October 25—and tests made of the soils for their ammonifying, nitrifying, and azofying powers. The yield of corn was secured from the plots examined.

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[1] Brown, P. E. Methods for bacteriological examination of soils. Media for quantitative determination of bacteria in soils. Iowa Agr. Exp. Sta. Research Bul. 11, p. 379–407. 1913.

[2] Löhnis, Felix, and Green, H. H. Methods in soil bacteriology. VII. Ammonification and nitrification in soil and solution. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, No. 19/21, p. 457–479. 1914.

Complete data obtained in this work have been given in another place,[1] and hence only summarized results are included here.

The results of the ammonification tests with dried blood and cottonseed meal are given in Tables I and II, respectively. The nitrification tests with ammonium sulphate and dried blood appear in Tables III and IV, and the azofication results are given in Table V.

TABLE I.—*Ammonification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	171. 11	220. 74	108. 76	110. 58
602	178. 07	231. 38	117. 86	116. 54
604	188. 82	243. 60	133. 43	131. 11
607	175. 22	229. 63	129. 78	124. 82
609	179. 96	238. 53	118. 53	116. 84
901	174. 75	232. 08	117. 04	114. 88

TABLE II.—*Ammonification of cottonseed meal on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	142. 01	163. 32	102. 13	111. 08
602	144. 54	168. 74	110. 09	122. 17
604	151. 18	177. 81	120. 18	126. 64
607	145. 49	168. 21	131. 11	123. 49
609	148. 50	171. 00	105. 78	119. 02
901	144. 07	165. 94	112. 73	115. 55

TABLE III.—*Nitrification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	12. 442	19. 883	11. 864	13. 797
602	15. 196	23. 311	14. 629	17. 433
604	20. 776	27. 087	18. 173	24. 032
607	15. 078	22. 884	16. 410	22. 211
609	18. 798	25. 226	13. 453	15. 048
901	13. 962	20. 713	12. 711	14. 014

[1] Brown, P. E. Bacteriological studies of field soils. II. The effects of continuous cropping and various rotations. Iowa Agr. Exp. Sta. Research Bul. 6, p. 211–246. 1912.

TABLE IV.—*Nitrification of ammonium sulphate on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	5.019	17.577	7.565	8.086
602	8.075	21.625	9.788	11.789
604	12.630	24.517	12.903	19.419
607	7.066	21.477	11.357	13.749
609	11.908	22.978	9.101	10.620
901	6.724	21.477	8.310	9.655

TABLE V.—*Azofication tests on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	9.50	3.93	13.52	10.32
602	17.46	15.07	19.92	17.52
604	20.64	18.25	23.12	20.72
607	14.27	17.46	20.72	18.32
609	18.25	15.87	18.32	16.72
901	14.27	11.88	16.72	15.12

The variations in the amount of moisture in the different plots at the same samplings were very small and the differences in bacterial activities which were found could not, therefore, be attributed to the different moisture conditions in the plots.

The yields obtained with corn on the various soils are given in Table VI, and comparing these with the ammonification, nitrification, and azofication results it will be noted that there is a remarkably good agreement.

TABLE VI.—*Yield of corn on rotation plots in 1911*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	35.5
602	2-year rotation	46.0
604	3-year rotation	50.7
607	2-year rotation; clover turned under	52.7
609	2-year rotation; cowpeas turned under	32.5
901	2-year rotation; rye turned under	43.2

The ammonification results with the dried blood and cottonseed meal did not always run exactly parallel, but the differences were slight, and in most cases the same comparisons were secured, so they need not be

considered separately. The same is true of the nitrification results with ammonium sulphate and dried blood.

Furthermore, the ammonification, nitrification, and azofication results are all in close agreement as to the relative effects on each of the various treatments; and, hence, the bacteriological results may be compared as a whole with the crop yields.

An examination of the tables reveals the fact that a greater crop yield was secured where the 2-year rotation was followed than on the continuous corn plot, and a still greater yield was secured where the 3-year rotation was followed. Exactly the same relations were found in the ammonification, nitrification, and azofication results.

Where the clover was introduced into the 2-year rotation as a green manure a greater crop yield was obtained than where it was not used. Furthermore, a slightly greater yield was obtained than on the 3-year rotation plot. The bacteriological results are not in accord with these differences; but in most cases the variations were not large, and the differences in crop yield were not great. Hence, the lack of agreement here should not be considered of great significance.

When cowpeas were used in the 2-year rotation, however, the yield was abnormally depressed. The bacterial activities were also depressed, but not to so great an extent. Evidently some unknown factor interfered here, as such a depression is hardly explainable. Where rye was turned under in the 2-year rotation the yield was less than on the regular 2-year rotation plot, and corresponding depressions were noted in the bacterial activities.

It is apparent that the ammonification, nitrification, and azofication results as a whole show a surprisingly close relation to the crop yield. Nitrification and ammonification tests frequently proceed in the same direction, and it is possible that after many confirmatory tests have been carried out it may be found that only one of these bacteriological tests of soils needs to be made. At the present time, however, the data available along this line are insufficient to warrant the interpretation of the results from one process as fitting another.

It is hardly expected, however, that azofication results will run parallel with ammonification and nitrification tests in any large number of studies. Conditions which favor the latter processes need not necessarily favor azofication.

These results as a whole, therefore, indicate that under normal soil conditions the ammonifying and nitrifying powers of soils may reflect fairly accurately their crop-producing power and show quite accurately the relative yields which will be secured. Only in special cases can similar dependence be placed on azofication results. These tentative conclusions have been further tested and are borne out by the later results.

TESTS ON ROTATION PLOTS IN 1912

The same series of plots was used in 1912 in the study of the relative effects of different rotations on bacterial activities and on crop production, but in some cases different individual plots were employed, as again only those which were cropped to corn were examined.

Ammonification tests were carried out by the dried-blood-air-dry-soil method with inoculum from fresh soil, the casein-fresh-soil method, and the dried-blood-fresh-soil method. The nitrifying power was tested by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. These methods were under investigation at the time of this study, and comparative tests of their efficiency have been reported in the work already referred to.[1]

Four samplings were made during the year—on August 9, August 19, October 7, and October 26. The variations in moisture content of the soils at the various dates were so slight that the differences observed could not be attributed to that factor, and the results of the determinations are not included here.

The crop yields were obtained from the plots as in the previous year.

The ammonification results appear in Tables VII, VIII, and IX, the nitrification results in Tables X and XI, and the crop yields are given in Table XII.

TABLE VII.—*Ammonification of dried blood in air-dry soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	148.33	54.93	124.78	122.42
603	157.55	66.31	130.27	127.92
605	170.69	79.77	138.71	138.71
608	172.65	82.40	141.85	143.42
610	168.53	75.73	136.95	130.67
902	151.27	64.15	125.17	119.09
904	161.08	71.61	131.06	138.21

TABLE VIII.—*Ammonification of dried blood in fresh soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	106.34	68.66	50.81	54.74
603	110.66	80.05	65.14	62.39
605	117.32	86.70	73.77	71.02
608	120.87	88.28	74.02	74.66
610	115.95	78.87	72.59	71.41
902	109.67	73.38	58.86	62.19
904	114.14	82.90	68.28	69.17

[1] Brown, P. E. Op. cit.

TABLE IX.—*Ammonification of casein on rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	61. 80	64. 84	58. 66	55. 33
603	67. 30	71. 80	65. 13	66. 31
605	71. 61	76. 52	68. 47	69. 45
608	72. 39	79. 07	70. 63	72. 79
610	68. 67	73. 37	67. 29	69. 25
902	62. 78	69. 06	63. 18	62. 39
904	67. 10	73. 37	67. 10	68. 67

TABLE X.—*Nitrification of ammonium sulphate in the air-dry soil of rotation plots in 1912*

Plot No.	Quantity of nitrates (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	10. 431	12. 443	8. 444	7. 232
603	13. 489	16. 751	12. 427	11. 333
605	15. 114	18. 941	15. 546	14. 557
608	15. 250	23. 931	16. 524	15. 250
610	14. 196	18. 110	15. 208	14. 733
902	12. 695	12. 893	9. 914	10. 936
904	14. 434	17. 410	14. 946	14. 686

TABLE XI.—*Nitrification of ammonium sulphate in the fresh soil of rotation plots in 1912*

Plot No.	Quantity of nitrates (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	11. 944	15. 300	7. 183	6. 844
603	12. 728	16. 601	10. 695	9. 776
605	14. 682	22. 583	12. 462	12. 154
608	15. 520	25. 078	13. 784	14. 224
610	13. 559	18. 264	12. 233	13. 999
902	11. 960	15. 837	7. 789	10. 629
904	13. 060	17. 414	10. 981	13. 166

TABLE XII.—*The yield of corn on rotation plots in 1912*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	50. 25
603	Corn and oats	63. 12
605	Corn, oats, and clover	69. 00
608	Corn and oats; clover turned under	74. 00
610	Corn and oats; cowpeas turned under	68. 50
902	Corn and oats; rye turned under	59. 50
904	Corn, corn, oats, and clover	67. 50

If these results are examined, it is found that practically uniform agreement was secured with the various methods—i. e., the relative ammonifying powers of the soils were the same whether the dried-blood or the casein method was employed, and it made little difference whether the dried-blood-air-dry-soil method was employed or the dried-blood-fresh-soil method was used. Similarly, in the case of nitrification, the same relative results were obtained whether the air-dry-soil method or the fresh-soil method was employed. It is unnecessary, therefore, to consider the results individually, and comparisons will merely be made between the bacterial results and the crop yields.

The largest crop yield was obtained in this year on the plot under the 2-year rotation with clover turned under. Similarly, the greatest ammonifying power and the greatest nitrifying power were found in this soil.. The soil under the 3-year rotation (corn, oats, and clover) was second in crop yield and in bacterial activities; the 2-year rotation with cowpeas as a green manure induced a slightly smaller crop yield and lower bacterial action; the 4-year rotation was still lower; the 2-year rotation (corn and oats) lower yet; the 2-year rotation with rye turned under gave a still smaller crop yield and lower bacterial action; and the continuous-crop plot was at the bottom of the list.

It is evident from these results that the ammonification and nitrification of nitrogenous organic material in soils and their crop-producing power are very closely related and that tests of the power of soils to produce ammonia or nitrates may be an indication of their crop-producing power, or at least of their relative crop-producing ability. Previous results are also confirmed regarding the similarity of the effects of soil treatment or ammonification and nitrification. Such need not always be the case, of course, as it is possible to conceive of conditions affecting the nitrifying organisms which do not similarly affect the ammonifiers, but it seems to be the case that in ordinary field conditions the two processes are quite similarly affected by treatment and probably only one process need be tested to gain some idea of the relative crop-producing power of soils.

TESTS ON ROTATION PLOTS IN 1913

The experiment on the same series of plots was continued in 1913, different individual plots being used for corn.

Three samplings were made during the season—on August 15, August 23, and August 26. Ammonification tests only were carried on, owing to the pressure of other work; and only one method, the casein-fresh-soil method, was employed. The crop yields were obtained as previously. Again, the moisture content of the soils at the different samplings varied so slightly that the differences may be considered negligible from the standpoint of the effects of treatment.

The results of the ammonification tests appear in Table XIII, and the crop yields are given in Table XIV.

TABLE XIII.—*Ammonification of casein on rotation plots in 1913*

Plot No.	Quantity of nitrogen.		
	Aug. 15.	Aug. 23.	Aug. 26.
	Mgm.	*Mgm.*	*Mgm.*
601	68. 38	60. 82	55. 67
602	71. 56	63. 47	59. 31
606	78. 74	69. 59	64. 05
607	74. 89	66. 35	63. 15
609	73. 53	64. 45	60. 52
901	75. 65	68. 15	63. 46
904	74. 28	65. 21	60. 97

TABLE XIV.—*Yields of corn on rotation plots in 1913*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	30. 0
602	2-year rotation: Corn and oats	53. 3
606	3-year rotation: Corn, oats, and clover	68. 0
607	2-year rotation: Corn and oats; clover turned under	64. 0
609	2-year rotation: Corn and oats; cowpeas turned under	60. 0
901	2-year rotation: Corn and oats; rye turned under	65. 3
904	4-year rotation: Corn, corn, oats, and clover	62. 6

Comparing the results, it is apparent that the indications of fertility given by the ammonification studies were borne out by the actual crop yields. The rank of the soils both in ammonifying power and in crop production was as follows:

Plot No.	Treatment.	Rank.
606	3-year rotation	1
901	2-year rotation; rye turned under	2
607	2-year rotation; clover turned under	3
904	4-year rotation	4
609	2-year rotation; cowpeas turned under	5
602	2-year rotation	6
601	Continuous corn	7

The results of these studies check those of previous years, therefore, and indicate that ammonification and crop production are very closely related and that the determinations of the ammonifying power of soils made during the growing season may show their relative crop-producing powers.

The plots in this series, as will be noted, ranked differently each year, both in crop yields and in bacterial activities, but it is not purposed to enter here upon a discussion of the reasons for such variations. The seasonal conditions, especially as regards rainfall, were undoubtedly of

prime importance. It will be noted, however, that the rotation of crops increased in every case both the crop yield and the bacterial activities. The use of green manure in the 2-year rotation sometimes proved more valuable than the 3-year rotation, and sometimes was of less value. This was probably due also to the moisture conditions. The point of importance here is, however, the fact that, regardless of seasonal conditions or of the effect on crops under particular conditions, bacterial activities and crop production were relatively similar.

TESTS ON MANURED PLOTS IN 1912

The manured plots were studied in 1912. Ammonification results were obtained by the casein-fresh-soil method, the dried-blood-air-dry-soil method, and the dried-blood-fresh-soil method; and nitrification tests were carried out by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. Four samplings were made during the season—on August 2, August 15, August 22, and September 9. The moisture conditions in the soils varied so slightly that they could not be considered of significance, and they are not included here. Crop yields were secured, corn being grown on the plots as in the other series.

Complete data from these experiments have been reported in another place [1] and only summarized results are given here.

The ammonification results are given in Tables XV, XVI, and XVII, the nitrification results in Tables XVIII and XIX, and the crop yields in Table XX.

TABLE XV.—*Ammonification of dried blood in the fresh soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	66. 90	83. 97	73. 57	66. 71
1005	84. 76	92. 21	83. 97	70. 63
1006	86. 32	106. 34	98. 88	85. 54
1007	97. 90	109. 47	98. 88	84. 95
1008	86. 72	95. 74	87. 50	76. 91

TABLE XVI.—*Ammonification of casein on manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	37. 87	68. 27	67. 49	51. 60
1005	46. 89	73. 57	72. 79	58. 86
1006	51. 79	77. 50	78. 87	66. 32
1007	51. 99	78. 48	79. 46	65. 72
1008	48. 78	75. 14	74. 75	60. 42

[1] Brown, P. E. Bacteriological studies of field soils. III. The effects of barnyard manure. Iowa Agr. Exp. Sta. Research Bul. 13, p. 421–448. 1913.

TABLE XVII.—*Ammonification of dried blood in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	80. 44	111. 83	106. 34	102. 81
1005	94. 76	117. 33	109. 47	117. 13
1006	100. 06	131. 25	122. 23	127. 92
1007	100. 85	137. 14	124. 00	133. 02
1008	95. 75	128. 90	113. 80	122. 62

TABLE XVIII.—*Nitrification of ammonium sulphate in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	8. 507	14. 794	12. 500	9. 211
1005	9. 326	15. 453	13. 693	10. 262
1006	10. 000	17. 710	14. 392	12. 593
1007	11. 655	18. 712	16. 401	12. 446
1008	10. 064	16. 696	14. 662	10. 444

TABLE XIX.—*Nitrification of ammonium sulphate in the fresh soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	5. 576	10. 946	10. 283	9. 141
1005	7. 259	12. 583	12. 543	10. 000
1006	8. 470	16. 733	14. 142	12. 698
1007	10. 282	18. 694	15. 641	13. 011
1008	8. 125	16. 164	12. 949	10. 528

TABLE XX.—*Yield of corn on manured plots in 1912*

Plot No.	Treatment.	Yield per acre.
		Bu.
1004	Check	50. 50
1005	8 tons of manure	77. 62
1006	12 tons of manure	86. 00
1007	16 tons of manure	87. 00
1008	20 tons of manure	81. 00

If the results secured in the ammonification tests are examined, it is seen that the effects of the manure were the same whatever method was employed. It is unnecessary, therefore, to consider the different results individually. Similarly in the case of nitrification, the fresh-soil and air-dry-soil methods yielded similar results, and general conclusions only need be drawn.

If the bacterial results as a whole are compared with the crop yields, it is found that there was exact agreement. Applications of manure increased the ammonifying and nitrifying powers of the soil, and the crop yield was also increased. Further gains in bacterial action and also in crop yields were obtained as the amount of manure applied was increased, but the maximum effect was obtained with the use of 16 tons of manure per acre. Beyond that point increasing the quantity of manure decreased both bacterial action and crop yields.

These results therefore check the previous observations that ammonification and nitrification tests may often run parallel. Previous results are also confirmed regarding the relation between crop yields and certain bacterial activities. Tests of the ammonifying power of soils or of their nitrifying powers apparently indicate quite accurately their crop-producing powers.

TESTS ON LIMED PLOTS IN 1911

The three plots in this series were sampled during 1911 on June 21, July 6, September 14, and October 24. Ammonification tests were made by the dried-blood and cottonseed-meal methods, nitrification by the ammonium-sulphate and dried-blood methods, and azofication by the mannite method. Crop yields were secured as in the other series studied. Complete results of these tests have been reported,[1] and only average results are given here.

The ammonification results appear in Tables XXI and XXII, the nitrification results in Tables XXIII and XXIV, the azofication results in Table XXV, and the crop yields in Table XXVI.

TABLE XXI.—*Ammonification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	207. 17	206. 60	128. 06	129. 78
509	208. 12	207. 30	144. 51	140. 05
508	214. 13	235. 22	155. 59	149. 32

[1] Brown, P. E. Bacteriological studies of field soils. I. The effects of lime. Iowa Agr. Exp. Sta. Research Bul. 5, p. 187–210. 1912.

TABLE XXII.—*Ammonification of cottonseed meal on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	131. 26	157. 22	126. 22	124. 32
509	132. 68	161. 06	141. 15	130. 28
508	142. 01	172. 58	151. 22	137. 90

TABLE XXIII.—*Nitrification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	13. 745	27. 056	20. 579	14. 570
509	15. 844	33. 857	23. 247	18. 434
508	21. 911	39. 686	29. 376	22. 946

TABLE XXIV.—*Nitrification of ammonium sulphate on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	8. 737	24. 987	14. 298	8. 762
509	10. 547	25. 475	20. 146	11. 743
508	14. 822	29. 034	24. 061	17. 890

TABLE XXV.—*Azofication tests on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	5. 52	2. 34	11. 89	11. 09
509	15. 07	16. 66	25. 41	27. 00
508	26. 21	30. 19	38. 93	37. 34

TABLE XXVI.—*Yield of corn on limed plots in 1911*

Plot No.	Treatment.	Yield per acre.
		Bu.
510	Check	52. 5
509	2 tons of lime	55. 0
508	3 tons of lime	55. 0

The ammonification results by the two methods employed were very similar, as also were the nitrification results; hence, these results need not be considered separately.

If the bacterial tests are compared with the crop yields, it is found that the lime increased ammonification, nitrification, and azofication in the soils, and the crop yield was similarly increased, the larger amount of lime bringing about the greater effect on the bacteria but exerting no further increasing effect on the crop grown.

These results as a whole therefore check those obtained on the plots under other methods of treament and show that bacterial transformations of nitrogenous compounds in the soil or, rather, the ability of soils to bring about the simplification of nitrogenous materials or the addition of nitrogen, may be considerably modified by various methods of soil treatment. Furthermore, they check previous results in showing that certain bacterial activities in the soil may be very closely related to the actual crop-producing power of the soil. The ammonifying power of soils, their nitrifying power, or even, in certain cases, their azofying power may therefore indicate the crop-producing power of soils or, at least, their relative crop-producing power.

CONCLUSIONS

(1) These experiments as a whole represent a line of investigation in soil bacteriology which it is believed will ultimately place the subject on a more practical basis—a basis which will permit the direct application of the results obtained to the solution of soil-fertility problems.

(2) The relations between the bacterial activities studied and the actual crop yields on these plots have proved so striking and so consistent that it was felt that accidental coincidence had been practically eliminated and the results might be considered to give a strong indication that certain bacterial activities in field soils are very closely associated with crop yields.

(3) Furthermore, the tentative conclusion presents itself that tests of such bacterial activities in the laboratory may indicate quite accurately the crop-producing power of a soil or, at least, the relative crop-producing power of several soils.

(4) If, further, more exhaustive tests confirm these preliminary observations, it may be possible to secure advance information regarding the crop-producing power of soils by means of laboratory tests of bacterial action in those soils.

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JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., FEBRUARY 7, 1916 NO. 19

AGGLUTINATION TEST AS A MEANS OF STUDYING THE PRESENCE OF BACTERIUM ABORTUS IN MILK

By L. H. COOLEDGE,

Research Assistant in Bacteriology, Michigan Agricultural Experiment Station

INTRODUCTION

In the investigation of the effect on milk of the diseases of the cow, with special reference to infectious abortion, it was found desirable to examine a large number of samples to determine whether or not *Bacterium abortus* Bang was being passed with the milk. The cultural and animal-inoculation methods were the only ones found available for this work.

The cultural method devised by Nowak [1] takes advantage of the fact that newly isolated cultures require an atmosphere partially depleted of oxygen. This atmospheric condition is obtained by growing the agar streaks from suspected material in a closed jar with *Bacillus subtilis*, having 1 sq. cm. of culture surface to each 15 c. c. of jar capacity. While the author has isolated *Bact. abortus* from milk sediment by this method, it is too tedious a process to apply to any number of samples. Plates are likely to be overgrown with colonies of fast-growing organisms, and the method has the further disadvantage of requiring several weeks to isolate and identify the cultures.

Evans [2] succeeded in isolating *Bact. abortus* from milk by plating on ordinary lactose agar to which 10 per cent of sterile blood serum was added just before plating. After incubating for four days, the colonies which developed were transferred to nutrient broth containing 1 per cent glycerin and to tubes of whole milk containing litmus.

The other method of study, the inoculation of guinea pigs with the milk, while more reliable, is far from satisfactory, owing to the fact that it takes 8 to 10 weeks for the lesions to develop, and it is probable that the organism must be present in large numbers to cause the characteristic lesions with the 5 c. c. of milk used for inoculation.

[1] Nowak, Jules. Le bacille de Bang et sa biologie. *In* Ann. Inst. Pasteur, t. 22, no. 6, p. 541–556, pl. 5–7. 1908.

[2] Evans, Alice C. Bacillus abortus in market milk. *In* Jour. Wash. Acad. Sci., v. 5, no. 4, p. 122–125. 1915.

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The other method of study, the inoculation of guinea pigs with the milk, while more reliable, is far from satisfactory, owing to the fact that it takes 8 to 10 weeks for the lesions to develop, and it is probable that the organism must be present in large numbers to cause the characteristic lesions with the 5 c. c. of milk used for inoculation.

[1] Nowak, Jules. Le bacille de Bang et sa biologie. *In* Ann. Inst. Pasteur, t. 22, no. 6, p. 541–556, pl. 5–7. 1908.

[2] Evans, Alice C. Bacillus abortus in market milk. *In* Jour. Wash. Acad. Sci., v. 5, no. 4, p. 122–125. 1915.

In studying the presence of *Bact. abortus* in milk it was found necessary to develop new technic in order to study a large number of samples. Knowing that this organism is sometimes present in considerable numbers in milk as it comes from the cow's udder, it was thought that this might indicate an infection of the udder and a consequent local production of antibodies. With this in mind, agglutination and complement-fixation tests were made, using milk and milk serum, instead of the usual method of using blood serum. *Bact. abortus* was used as antigen. The object of this paper is to report upon this method.

TECHNIC EMPLOYED

COMPLEMENT-FIXATION TEST.—The complement-fixation test as used by Surface [1] and others, was employed in this work. Rennet milk serum was used in the following quantities: 0.1, 0.04, 0.02, and 0.005 c. c. Milk was considered positive only when the tube containing 0.04 c. c. of serum was positive. Preliminary tests run upon samples of milk show that the agglutination and complement-fixation tests correspond closely. For this reason only the results of agglutination tests will be given in this paper.

AGGLUTINATION TEST.—Antigen was prepared for the agglutination test by growing a culture of *Bact. abortus* upon ordinary agar for 48 hours. The growth was then washed off with a solution containing 0.9 per cent sodium chlorid and 0.5 per cent phenol. The suspension was then filtered through a coarse filter paper and standardized so that the turbidity compared with tube 1.5 of McFarland's nephelometer.[2] Four c. c. of this bacterial suspension are placed in each of the small test tubes used and the following quantities of milk added: 0.1, 0.05, 0.025, 0.01, and 0.005 c. c. In this way approximate dilutions of 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000 were obtained. It was found that turbidity due to the whole milk added did not interfere with the reading of the reaction. When a dilution lower than 1 to 50 was made, rennet milk serum was used.

For the experiment given in Table I, a cow was selected whose milk had given a negative agglutination reaction since first tested, October 10, 1914, using *Bact. abortus* as antigen. Thirty-five c. c. of a 48-hour broth culture of *Bact. abortus* was introduced into the right rear quarter after it had been milked dry. As shown in the table, the agglutinins had appeared in the right rear quarter the following day and soon spread to the other quarters. This spreading was probably brought about by the organism being carried from quarter to quarter upon the hands during milking. After the cow freshened the reaction was seen to gradually die out.

[1] Surface, F. M. The diagnosis of infectious abortion in cattle. Ky. Agr. Exp. Sta. Bul. 166, p. 301–365, 5 fig. 1912.

[2] McFarland, Joseph. The nephelometer. . . . *In* Jour. Amer. Med. Assoc., v. 49, no. 14, p. 1176–1178, 2 fig. 1907.

TABLE I.—*Test showing the appearance in milk of agglutinins for Bacterium abortus after the introduction into the cow's udder of a pure culture of Bact. abortus Bang* [a]

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914.																				
Oct. 10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1915.																				
Feb. 8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
25	(b)	(b)	(b)	(b)	(b)															
26	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
27	P	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mar. 1	+	+	+	−	−	+	P	−	−	−	+	P	−	−	−	+	P	−	−	−
2	+	+	−	−	−	+	P	P	−	−	+	+	+	P	−	+	P	P	−	−
5	+	+	−	−	−	+	+	P	−	−	+	+	+	P	−	+	+	P	−	−
8	+	+	+	−	−	+	+	+	P	−	+	+	+	−	−	+	+	+	−	−
9	(c)	(c)	(c)	(c)	(c)															
10	+	P	−	−	−	+	+	−	−	−	+	P	−	−	−	+	P	−	−	−
11	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Apr. 17	+	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
May 26	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
June 4	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
July 28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Aug. 21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sept. 13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] The +, −, and P signs used in all the tables refer to agglutination reaction in the corresponding tube. For instance, +++P− indicates that agglutination took place in the tubes containing 0.1, 0.05, and 0.025 c. c. of milk, partial agglutination took place in the tube containing 0.01 c. c. of milk, and there was no agglutination in the tube containing 0.005 c. c. of milk.

In all cases, unless otherwise stated, the milk was taken a little before what was estimated to be the middle of the milking.

[b] 35 c. c. of a 48-hour broth culture of *Bact. abortus* introduced into right rear quarter.

[c] Cow calved. Bull calf died on Mar. 13, 1915, owing to undigested curd. Reaction of blood of calf; −agglutination; +complement-fixation test.

Table II gives the history of milk from a cow with a record of frequent abortions. As shown in the table, the isolation of *Bact. abortus* from the milk and the results of guinea-pig inoculation prove the presence of this bacterium, as indicated by agglutination reactions.

TABLE II.—*History of milk from a cow with a record of frequent abortions* [a]

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.01 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914.																				
Jan. 10 [b]																				
Apr. 30	+	+	+	−	−	+	+	P	−	−	+	+	+	P	−	+	+	+	+	−
May 5	+	+	+	−	−	+	+	P	−	−	+	+	−	−	−	+	+	+	−	−
June 20	+	+	+	P	−	+	+	+	−	−	+	+	+	P	−	+	+	+	P	−
July 11	+	−	−	−	−	+	P	−	−	−	+	+	+	P	−	+	P	−	−	−
12 [c]																				
Aug. 10	+	+	−	−	−	+	+	+	P	−	+	+	+	P	−	+	+	+	P	−
28	+	+	−	−	−	+	−	−	−	−	+	+	−	−	−	+	+	−	−	−
Oct. 10	+	+	−	−	−	+	+	−	−	−	+	+	+	−	−	+	+	+	−	−
31	+	+	P	−	−	+	+	+	−	−	+	+	+	P	−	+	+	+	−	−
Nov. 19 [d]																				

[a] Known abortions: Dec., 1909; Jan., 1914. Jan., 1911, last living normal calf. Other records of abortions lost.

[b] Isolated a pure culture of *Bact. abortus* direct from milk.

[c] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed eight weeks later.

[d] Died; impaction of stomach. No lesions or abnormal conditions found in udder.

In Table III the record of milk from another cow is given. Here again we have positive agglutination coupled with abortions and milk shown to contain *Bact. abortus* by guinea-pig inoculation.

TABLE III.—*History of milk from a cow with a record of frequent abortions*

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914.																				
July 20	+	P	−	−	−	+	+	P	−	−	+	+	−	−	−	+	P	−	−	−
Aug. 4 [a]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
10	+	+	−	−	−	+	+	−	−	−	+	+	+	−	−	+	+	−	−	−
24	+	+	+	−	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	−
Oct. 1	+	+	+	−	−	+	+	P	−	−	+	+	+	−	−	+	+	+	−	−
Nov. 27 [b]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dec. 2	+	+	+	−	−	+	+	+	−	−	+	+	+	−	−	+	+	+	−	−
1915.																				
Jan. 15	+	+	P	−	−	+	P	−	−	−	+	P	−	−	−	+	−	−	−	−
Mar. 25	P	P	−	−	−	P	P	−	−	−	P	P	−	−	−	P	P	−	−	−
June 8	P	P	−	−	−	P	P	−	−	−	P	−	−	−	−	P	−	−	−	−
30 [c]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Sept. 10 [d]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Oct. 10	+	+	P	−	−	+	+	P	−	−	+	+	P	−	−	+	+	P	−	−
Nov. 4	+	P	−	−	−	+	P	−	−	−	+	+	P	−	−	+	+	P	−	−

[a] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed 10 weeks later.

[b] Aborted a 7-month fetus.

[c] Right rear quarter, positive guinea-pig inoculation. Right front quarter lost, and left rear and left front quarters negative.

[d] Aborted a 7-month fetus.

In Table IV is given the record of milk from a cow that has never aborted. On June 16, 1915, agglutinins had appeared in all but the left front quarter. Guinea-pig inoculations made on June 30 were positive for infectious abortion in all but the left front quarter. On October 16, 1915, the reaction had spread to the left front quarter. Milk from this quarter is now being tested by guinea-pig inoculation.

TABLE IV.—*History of milk from a cow that has never aborted*

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1915.																				
Apr. 9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
June 16	+	P	−	−	−	P	P	−	−	−	+	P	−	−	−	−	−	−	−	−
30 [a]	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Oct. 16	+	+	P	−	−	+	+	+	−	−	+	+	−	−	−	+	+	+	+	−

[a] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions and blood reactions, with exception of left front quarter, which was normal.

While, in Tables I to IV, a positive agglutination test points to the presence of *Bact. abortus* in the milk, this is not always true if judged by guinea-pig inoculation. In several cases the writer was unable to get positive lesions in guinea pigs with milk from all four quarters that gave a positive agglutination reaction. In these instances it is probable that the agglutinins were coming from the blood stream, or, if due to a bacterial invasion of the udder, the bacterium may have been present in too small numbers to cause lesions in guinea pigs with the 5 c. c. of milk used for inoculation. In the instances of agglutination with negative guinea-pig inoculation it was noticed that the reaction from quarter to quarter seemed to be fairly constant. In the tables given, the reaction is seen to vary a good deal from quarter to quarter. This, the writer believes, indicated that in the cases of reaction without pathogenicity to guinea pigs the agglutinins were coming to each quarter from a common source the blood.

Though many samples of milk have been inoculated into guinea pigs, at no time has a sample been found with a negative agglutination test that would produce the typical lesions of infectious abortion.

The present value of this test is that it enables one to select from a herd the cows whose udders may be infected with *Bact. abortus*. The comparatively small number separated by this method may then be examined by guinea-pig inoculation and cultural methods.

If *Bact. abortus* is found to be pathogenic for humans, as has been suggested by Melvin,[1] this test may be of value as another means of safeguarding certified and all unpasteurized milk.

From observations and tests now being made it appears that it may be possible to differentiate samples in which the agglutinins come from the blood from those in which the agglutinins are produced in the udder.

SUMMARY.

A pure culture of *Bacterium abortus* Bang introduced into the milk cistern of a cow's udder caused the appearance of agglutinins in the milk.

In every case in which *Bact. abortus* was found present in the milk by animal inoculation the agglutinins for this organism were also found, but this bacterium was not found in every case in which agglutinins were demonstrated.

The agglutination test is of value in studying the presence of *Bact. abortus* in milk when it is desired to study a large number of samples.

If *Bact. abortus* is found to be pathogenic for humans, this test may be of value as another means of safeguarding certified and all unpasteurized milk.

[1] Melvin, A. D. Infectious abortion of cattle and the occurrence of its bacterium in milk. I.—Introductory statement. *In* U. S. Dept. Agr. Bur. Anim. Indus. 28th Ann. Rpt. 1911, p. 137–138. 1913.

BORON: ITS ABSORPTION AND DISTRIBUTION IN PLANTS AND ITS EFFECT ON GROWTH

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INTRODUCTION

The experiments reported in this paper were made in connection with a cooperative study of borax and calcined colemanite as larvicides for the house fly conducted by the Bureaus of Entomology, Chemistry, and Plant Industry, of the Department of Agriculture. The object of this particular study was to determine the effect of boron-treated horse manure on plant growth and to study the absorption of boron and its distribution in the roots, stems, and fruit of plants grown on soil fertilized with this manure and on soil fertilized with untreated manure. The plants were grown in pots in the greenhouses of the Department and on open plots at Arlington Experimental Farm, Va.; Dallas, Tex.; Orlando, Fla.; and New Orleans, La. Analyses of the soil from several treated and untreated plots are included.[1]

Certain deposits of boron have been known for centuries, but the wide distribution of this element in mineral and vegetable matter has been recognized only during the last few years. Probably the first to record the presence of boron in plants were Wittstein and Apoiger (14),[2] who found it in the seeds of *Maessa picta*. Since then many observers have found boron in soils, rocks, fruits, and vegetables.

As soils in many places contain boron, it is not surprising that this element is widely distributed in small amounts in plants. It is also probable that boron is present in nearly all animal material. Bertrand and Agulhon (3) report its presence in the hair, horns, bones, liver, and muscles of animals. They detected boron in 27 species of animals, and state that it probably exists in all animals, being more common in those of marine origin. Boron was also found in human, asses', and cows' milk and in the eggs of the chicken, turkey, and goose.

The toxic effect of boron on plants was first shown in 1876 by Peligot (12), who noted a yellowing of the leaves of beans and reported that in many cases the yellow leaves fell from the plants. The previous year Heckel (8) reported that 1.25 per cent solutions of alkali borate retarded germination for from one to three days, and that 3 per cent of the alkali borate solutions stopped germination entirely. Loew (10, p. 374) states

[1] The writer desires to express his thanks to Mr. W. D. Hunter, of the Bureau of Entomology, for his material assistance in arranging for the experiments in the South.

[2] Reference is made by number to "Literature cited," pp. 889–890.

that certain algæ, such as Spirogyra and Vaucheria, are resistant to the action of boron. Morel (11), however, states that very weak solutions of boric acid arrest the development of lower fungi and similar organisms. He suggests that boric acid may be used, like copper, to attack such diseases as mildew and anthracnose. The effect of boron on the lower plants, fungi, yeasts, etc., has been but little studied.

Agulhon (1) and Bertrand (2) have stated that boron in small amounts acts as a stimulant to plant growth. Pellet (12) calls attention to some experiments which indicate that compounds of both manganese and boron, singly and combined, have no effect on the growth or yield of the sugar beet. He concludes that the results of other workers claiming a stimulation are too few and are untrustworthy.

Many investigations regarding the effect of boron on plants and plant growth have been reported, but no attempt to review all such experiments is made in this paper. For a review of this subject the publication of Haselhoff (7) and the recent work of Brenchley (4), where various inorganic plant poisons and stimulants are discussed, should be consulted.

EXPERIMENTAL WORK

Very few of the previous studies have included a quantitative estimation of the boron present in plants, and no experiments concerning the effects of calcined colemanite (crude calcium borate) on plant growth have been reported. As both borax and calcined colemanite are valuable larvicides for the house-fly maggot, it seems advisable to determine the effect of manure treated with both borax and calcined colemanite on the growth of a variety of plants.

The manure used in these tests was treated with the amounts of borax or calcined colemanite noted in the tables, and stood in the open for 10 days before it was applied to the soil. For the plot tests, the manure was applied at the rate of 20 tons per acre and was then plowed under, the ground harrowed, and sometimes rolled and reharrowed, before planting. In nearly all of these experiments borax or calcined colemanite was applied to the manure in larger quantities than were required to act as a larvicide—i. e., 0.62 per pound per 8 bushels, or 10 cubic feet. When the manure was mixed with the soil at the rate of 20 tons per acre, 216 pounds of borax per acre were present. Furthermore, the manure was not allowed to stand and leach for longer than 10 days; consequently, practically the entire amount of borax added reached the soil.

When 0.62 pound of borax was applied to each 8 bushels of manure and the weight of 8 bushels of manure estimated at 115 pounds (the average weight of fresh manure containing a large amount of straw), 100 pounds of manure contained 0.54 pound of borax, and when the manure was applied to the soil at the rate of 1 part to 40, the percentage of boron in the soil, calculating the weight of 1 acre of soil 6 inches deep as 1,750,000 pounds, was 0.0015.

Tests with tomato (*Lycopersicon esculentum*) and lettuce (*Lactuca sativa*) were made on plants which had been grown in boxes in greenhouses until they were 2 to 3 inches high, when they were transplanted in their respective pots containing the mixtures of manure and soil. The potatoes (*Solanum tuberosum*) tested were of the Green Mountain variety and the seeds used in growing the other plants were common varieties. The percentages of boric acid as recorded in the tables are calculated to a water- and ash-free basis. At least four pots for each treatment were employed in the pot tests. The plots at Arlington Farm were one-twentieth of an acre and those in the South about one-sixtieth of an acre in size. The tests with lettuce were carried out in benches, each 3 by 5 feet.

DESCRIPTION OF METHODS

Many tests for determining boron in foods and other material have been devised. When small amounts are present, as was the case in the present experiments, it is determined colorimetrically, using curcumin, the active principle in turmeric (*Curcuma longa* L.), a characteristic red color being given when boron is present.

In preparing the samples, the roots were separated from the plants. Both roots and plants were washed, dried, and cut into small pieces for analysis. In some cases the fruit also was tested. In such instances it was washed, dried, and ground for analysis. Boron was determined by the use of freshly prepared strips of curcumin paper, prepared by immersing large unfolded filter paper in a 0.2 per cent alcoholic solution of curcumin. The procedure was as follows: About 3 gm. of a dried sample were treated with sufficient saturated lime water to make the reaction alkaline. After a thorough mixing in platinum dishes, the samples were dried and heated in a muffle until all of the organic matter had burned off. Ten c. c. of water and a little hydrochloric acid were added and the solution was warmed, filtered, washed, and made to 100 c. c. volume. A 50 c. c. aliquot was usually taken for the determination of the boron, but this varied according to the amount present. To the 50 c. c. aliquot, or a smaller aliquot diluted to 50 c. c., placed in small porcelain evaporating dishes, 2 c. c. of hydrochloric acid were added, and strips of curcumin paper were suspended and allowed to dip into those solutions to the depth of one-fourth of an inch. In all cases standard boric-acid solutions, as well as blanks, were simultaneously employed. After four hours the colors on the strips of paper were compared and the percentage of boric acid determined.

In the case of soils, the boron soluble in weak hydrochloric acid, not the total boron, was determined. Fifty gm. of soil were shaken with 200 c. c. of a solution of hydrochloric acid (1:20) for one hour. This was filtered and 100 c. c. of the filtrate made alkaline with lime water, evaporated to dryness, and ashed. The ash was acidified with hydrochloric

acid and the solution made to 100 c. c., a 50 c. c. aliquot being used for the colorimetric test. In some cases larger amounts of soil were used for the tests. From 2 to 3 gm. of the plant samples were used for moisture and ash determinations.[1]

RESULTS OF EXPERIMENTS

The results of the experiments are expressed in all the tables and text as percentages of boric acid. Some analyses of boron soluble in weak hydrochloric-acid extracts of soils are also reported. The form of the combination of the boron in plants is not known. The boron of soils is in part present in insoluble combinations with silica, and the absence of acid-soluble boron in some soils may be thus explained. Ash results are also reported for most of the plants analyzed. Separate analyses of the tops, roots, and fruits are tabulated.

In Table I analyses showing the distribution of ash and boron in the tops and roots of wheat (*Triticum* spp.) and beets (*Beta vulgaris*) 3 months old, grown in the presence of calcined colemanite and borax, with and without the addition of lime, are recorded. More boron was found in the tops than in the roots of both plants. The beets absorbed more boron than the wheat plants, especially from the soil treated with calcined colemanite. All of the control plants contained a little boron.

TABLE I.—*Percentage of boron in wheat and beets: Greenhouse pot tests*[a]

Series No.	Treatment of manure per 8 bushels.	Wheat (dry basis).				Beets (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.
1	0.75 pounds of calcined colemanite added	*Per ct.* 15.55	*Per ct.* 0.0103	*Per ct.* 20.00	*Per ct.* Trace.	*Per ct.* 23.10	*Per ct.* 0.0315	*Per ct.* 17.74	*Per ct.* 0.0020
2	1.5 pounds of calcined colemanite added	12.96	.0097	24.76	Trace.	21.69	.0402	12.75	.0025
3	1 pound of borax added	12.58	.0097	33.48	0.0008	22.39	.0120	14.52	.0054
4	1 pound of borax and 1 ounce of lime added	8.51	.0122	23.39	.0029	23.07	.0172	.....	.0097
5	1 pound of borax and 3 ounces of lime added	9.63	.0105	25.69	.0044	22.77	.0154	14.12	.0087
6	1 pound of borax and 9 ounces of lime added	11.07	.0173	26.24	Trace.	20.36	.0062	14.41	.0047
7	Control	9.20	.0013	23.76	Trace.	23.80	Trace.	14.56	.0013

[a] Forty parts of soil and 1 part of boron-treated manure were mixed in all the pot and bench tests.

A similar series of tests using tomatoes and cowpeas (*Vigna sinensis*) are recorded in Table II. The number and weight of the tomatoes obtained from four pots, which are also recorded, show the injurious

[1] The analyses were completed with the assistance of Mr. J. B. Wilson, of the Animal Physiological Chemical Laboratory, to whom the writer desires to express his indebtedness.

action of the boron alone and the benefit derived from adding lime. The tops of the tomatoes contained rather a large quantity of boron, the roots and fruit but traces. More boron was absorbed by the tomato plants when borax was added than with the addition of calcined colemanite. The addition of lime with the borax retarded the absorption of boron. The lowest percentage of dry matter was found in the tomatoes grown on the soil where borax alone was added. The tops of the control plants contained the least ash.

TABLE II.—*Boron in tomatoes and cowpeas: Greenhouse pot tests*

Series No.	Treatment of manure per 8 bushels.	Tomatoes.							
		Tops.		Roots.		Fruit.		Yield of fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (ash-free basis).	Number.	Weight.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per cent.*		*Ounces.*
1	0.75 pound of calcined colemanite added.	13.13	0.0054	9.59	Trace.	6.04	Faint trace.	17	37.25
2	1.5 pounds of calcined colemanite added.	14.44	.0107	10.86	...do...	5.94	.....do.....	16	31.5
3	1 pound of borax added.....	11.87	.0146	28.89	None..	4.72	.....do.....	10	10
4	1 pound of borax and 1 ounce of lime added.	12.95	.0123	13.76	...do...	.....	.....	15	33
5	1 pound of borax and 3 ounces of lime added.	12.15	.0072	10.66	...do...	5.26	Faint trace.	17	34
6	1 pound of borax and 9 ounces of lime added.	12.00	Trace.	19.43	...do...	5.85	.....do.....	18	35
7	Control.....	10.12	...do...	21.88	Trace.	5.92	.....do.....	23	40.25

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).					
		Tops.		Roots.		Fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0.75 pound of calcined colemanite added.....	9.27	0.0339	18.52	0.0033	3.68	0.0135
2	1.5 pounds of calcined colemanite added.....	9.25	.0287	27.04	Trace.	3.90	.0106
3	1 pound of borax added.....	8.54	.0242	24.40	None.	3.36	.0133
4	1 pound of borax and 1 ounce of lime added.....	10.96	.0115	10.01	....do.....	4.12	.0222
5	1 pound of borax and 3 ounces of lime added.....	10.08	.0237	17.44	....do.....	3.01	.0097
6	1 pound of borax and 9 ounces of lime added.....	11.36	.0302	20.64	.....	3.40	.0029
7	Control.....	7.84	.0068	22.58	None.	3.20	.0094

The tops of the cowpeas contained the most boron and the roots the least, the fruit being intermediate. The addition of lime with the borax did not influence the total amount of boron absorbed by the plants. The control cowpeas contained larger amounts of boron than the tomato control plants. The tops of the control cowpeas contained the least ash.

The results of the greenhouse, bench, and pot tests with lettuce and tomatoes are recorded in Table III. It is evident that the lettuce plants took up boron in proportion to the amounts present in the soil. The control lettuce contained the lowest percentage of solids and indicated the presence of boron. A slight chlorosis of the lettuce plants grown in series 1 and 2 was seen, but no injury to the roots was observed. The results of the analyses of the upper and lower 6 inches of soil in the benches show an even distribution of the boron.

TABLE III.—*Boron in lettuce and tomatoes: Greenhouse bench and pot tests*

Series No.	Treatment of manure per 8 bushels.	Lettuce (entire plant).		Soluble boron as boric acid in soil on which lettuce was grown.	
		Dry matter.	Boron as boric acid (dry basis).	Upper 6 inches of soil.	Lower 6 inches of soil.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	0.75 pound of borax added	11.6	0.00036	0.0012	0.0010
2	1.25 pounds of borax added	10.0	.00064	.0022	.0028
3	Control	9.0	.00020	Faint trace.	Faint trace.
4	0.5 pound of borax added	.....	.00036	.....	.....
5	0.62 pound of borax added	.....	.00042	.....	.....
6	0.75 pound of borax added	.....	.....	.....	.....
7	Control	.....	.00015	.....	.....

Series No.	Treatment of manure per 8 bushels.	Tomatoes.					
		Tops (dry basis).		Fruit (fresh basis).		Yield.	
		Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (water and ash free basis).	Number.	Weight.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*		*Ounces.*
1	0.75 pound of borax added	12.98	0.0089	6.55	Faint trace.	123	157
2	1.25 pounds of borax added	12.94	.0196	6.60	do.....	101	139¾
3	Control	10.10	.0009	6.75	.....do.....	120	159½
4	0.5 pound of borax added	10.01	.015	8.10	0.0002	.....	.....
5	0.62 pound of borax added	10.77	.016	8.01	.0004	.....	.....
6	0.75 pound of borax added	7.72	.....	7.51	.0003	.....	.....
7	Control	7.72	.0015	8.00	.....	.....	.....

Tomato plants 1, 2, and 3, Table III, were 6 months old at the time of analysis. The yield of fruit from three pots in each series, 1, 2, and 3, showed no reduction in the case of the 0.75-pound borax application, but the 1.25-pound borax application reduced the yield. The dry matter of the control fruit, series 3, is higher than in series 1 or 2, and the ash of the control tops, series 3, is lower than the ash for the tops, series 1 and 2. The tomato plants, series 4, 5, 6, and 7, were younger and smaller than those of series 1, 2, and 3. In all the tomato plants (Table III) the tops contained practically all the boron, the fruit showing only traces.

The results with wheat grown in plots at Arlington Farm, Va., are given in Table IV. The manure was applied at the rate of 20 tons per acre. The wheat was planted in October, 1913, and harvested in June, 1914, the soil samples being tested at the time of harvesting. On the borax plot the wheat plants which were yellow during the winter, became green and normal in appearance in the spring. The yield of wheat from the borax plot was 90 per cent of the control, but larger than that from an unmanured plot which was simultaneously tested. The amount of borax added to the borax plot was about four times that necessary to act as a larvicide, but only a trace of boron was found in the wheat grain or straw. The wheat grains were sound and the nitrogen and ether-extract results of the control differed very little from those of the wheat and straw from the borax-treated plot. A trace of boron was found in the grains and straw from the borax plot, and the borax-treated soil showed 0.003 per cent of boric acid. The soil sample from the borax plot contained more nitrates than the control sample. Nitrogen was estimated by the Kjeldahl-Gunning method, and nitrates by the method recommended by the American Public Health Association.

TABLE IV.—*Percentage of boron in wheat, straw, and soil: Plot tests at Arlington Farm, Va.*

Series No.	Treatment of manure per 8 bushels.	Material.	Nitrogen.	Nitrogen as ammonia (MgO method).	Nitrogen as nitrates.	Ether extract.	Acid-soluble boron as boric acid.
1	2 to 3 pounds of borax added.	Wheat grains	2.15	.....	.....	1.70	Faint trace.
		Wheat straw	.281	.....	.....	2.12	Do.
		Soil 3 to 4 inches deep	.09	0.004	0.0018	.....	0.003.
2	Control	Wheat grains	2.21	.....	.....	1.77	None.
		Wheat straw	.323	.....	.....	2.27	Do.
		Soil 3 to 4 inches deep	.09	.003	.0012	.....	Do.

Results of the analyses of soybeans (*Glycine hispida*), string beans (*Phaseolus vulgaris*), and potato plants grown on plots at Arlington Farm, Va., are recorded in Table V. The roots and beans of the soybeans contained about equal amounts of boron, and rather large quantities were found in the tops of all the plants analyzed. There was a more equal distribution of boron in the roots, tops, and beans of the string beans than in the case of the soybeans.

The potatoes showed only traces of boron in the tops, the largest part of the boron being found in the roots, although the tubers contained a fairly large amount. All control plants contained a little boron. The addition of lime with the borax did not prevent the absorption of boron by the plants, as much boron being absorbed from the calcined-colemanite plots as from the borax plots.

TABLE V.—*Percentage of boron in soybeans, string beans, and potatoes: Plot tests at Arlington Farm, Va.*

Series No.	Treatment of soil per square rod.	Boron as boric acid (dry basis).								
		Soybeans.			String beans.			Potatoes.		
		Roots.	Tops.	Beans.	Roots.	Tops.	Beans.	Roots.	Tops.	Potatoes.
1	1.61 pounds of calcined colemanite added	0.0086	0.0048	0.0092	0.0044	0.0075	0.0045	0.0170	0.0012	0.0094
2	2.88 pounds of calcined colemanite added	.0160	.0076	.0136	.0083	.0177	.0213	.0144	Trace.	.0021
3	3.96 pounds of borax added	.0124	.0047	.0104	.0086	.0093	.0117	.0354	..do...	.0066
4	3.96 pounds of borax and 2 pounds of lime added	.0126	.0040	.0164	.0093	.0099	.0080	.0165	..do...	.0019
5	2 pounds of lime added.	.0030	.0008	.0036	.0050	None.	.0042	Trace.	None.	.0010

In Table VI results of the analyses of corn (*Zea mays*), wheat, peas (*Pisum sativum*), and oats (*Avena sativa*), grown on plots at New Orleans, La., and Dallas, Tex., are recorded. The entire plants, which were 3 months old and small, were used. The corn and wheat plants took up equal amounts of boron. Soluble boron was found in all nine samples of soil from New Orleans, while only two of the five samples from Dallas contained any. The peas absorbed more boron than the oats, especially in series 1, 2, and 3.

TABLE VI.—*Percentage of boron in corn, wheat, peas, oats, and soil: Plot tests at New Orleans, La., and Dallas, Tex.*

Series No.	Treatment of manure per 8 bushels.	New Orleans, La.			Dallas, Tex.		
		Boron as boric acid (entire plant, dry basis).		Soluble boron as boric acid in soil, sample taken 4 inches deep.	Boron as boric acid (entire plant, dry basis).		Soluble boron as boric acid in soil, sample taken 3 inches deep.
		Corn.	Wheat.		Peas.	Oats.	
1	0.5 pound of borax added	0.013	0.011	0.0006	0.010	0.001	0
2	0.62 pound of borax added	.015	.015	.0009	.010	006	Trace.
3	Control	Trace.	Trace.	Trace.	.003	0	0
4	0.75 pound of borax added	.....	.....	.0003	.040	.025	0
5	1.25 pounds of borax added	.....	.....	.0013	.026	.025	Trace.
6	Control	.....	.....	Trace.	.0005	0	0
7	0.75 pound of calcined colemanite added	.....	.....	.0008	.....	.....	.....
8	1.50 pounds of calcined colemanite added	.....	.....	.0032	.....	.....	.....
9	Control	.....	.....	Trace.	.....	.....	.....

TABLE VII.—*Percentage of boron and ash in radishes, string beans, cowpeas, peas, and soil: Plot tests at Orlando, Fla.*

Series No.	Treatment of manure per 8 bushels.	Radishes (dry basis).				String beans (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
1	0.75 pound of borax added	34.44	0.162	50.08	0.039	.....	0.086	22.98	0.011
2	1.25 pounds of borax added	49.49	.226	51.12	.087	17.56	.080	14.89	.015
3	Control	45.25	.018	45.04	.010	22.80	.011	.....	.007

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).				Peas (entire plant, dry basis).	Soluble boron as boric acid found in sample of soil 3 to 4 inches deep.
		Tops.		Roots.			
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Boron as boric acid.	
1	0.75 pound of borax added	29.49	0.162	35.15	0.222	0.212	0.0006
2	1.25 pounds of borax added	33.22	.140	45.68	.240	.229	.0010
3	Control	20.18	.024	.....	.029	.024	.0003

In Table VII the boron content of radish (*Raphanus sativus*), string-bean, cowpea, and pea plants, grown on borax and control plots at Orlando, Fla., is given. An appreciable amount of soluble boron was found in the soil samples from all three plots. The radish plants contained a large amount of boron in the tops, as well as an appreciable quantity in the roots. The string beans did not absorb as much boron as the radishes, but contained a large percentage of the absorbed boron in the tops. The cowpeas absorbed large amounts of boron, more being found in the roots than in the tops. The pea plants also absorbed boron in great quantities. All the control plants contained boron to a marked degree, which is not surprising, as 0.0003 per cent of soluble boron was found in the control soil sample examined at the close of the test.

As there was little rain at Orlando while these tests were being conducted, and as relatively large quantities of soluble boron were found in the samples of soil tested, it is not surprising that the plants absorbed large amounts of boron.

DISCUSSION OF EXPERIMENTAL WORK

It apparently made little difference in the quantities of boron absorbed by the various plants whether it was added to the manure used on the soil in the form of calcined colemanite or as borax. The addition of

lime to the borax also showed no definite action in preventing the absorption of boron, although with beets (Table I) and with one series of tomatoes (Table II) such a reduction is indicated where the largest application of lime was made. Most of the plants analyzed took up boron in proportion to the amounts present in soluble form in the soil.

The leguminous plants, which were most easily injured by boron, absorbed larger amounts than the other plants tested, while wheat and oats absorbed but little boron. It is particularly noteworthy that the wheat grown at Arlington Farm, Va., on soil fertilized with manure heavily treated with boron showed only traces of boron in the grain and straw. Haselhoff (7) found boron in the stalk of maize, but not in the grain.

The most striking differences in the absorption and distribution of boron are shown by the leguminous plants, where a more even distribution between roots, tops, and fruit is found. Potatoes also showed rather a large quantity of boron in the roots and tubers, but only a small amount in the tops. Succulent plants like beets also absorbed boron. On the other hand, tomatoes and wheat showed only traces of boron in the fruit and but little in the roots. Agulhon (1) has investigated the action of boric acid on wheat, using synthetic sterile liquid media, including both soil and water cultures. He recommends 0.0012 per cent of boron to obtain the best growth. In these tests, when borax was added at the rate of 0.62 pound to each 8 bushels of manure and this manure applied to the soil at the rate of 15 tons per acre, 0.0015 per cent of boron was added to the soil.

The fact that all control plants contained a little boron shows the wide distribution of boron in the soil. From the large amounts taken up by the control plants grown at Orlando, Fla., it appears that the soil there contains more than the soil at Dallas, Tex., New Orleans, La., or Arlington Farm, Va.

The ash results of the various portions of the plants analyzed vary considerably, and the variations are not in a definite direction.

A spotting or yellowing of the leaves of plants, which was first noted by Hotter (9) and later reported by several investigators, was observed in these experiments when boron was present in the soil to any extent. In the case of the tomato plants, Table II, a yellowing of the leaves was noted when borax was used at the 0.75-pound rate, but the yield was unaffected. In some of the legumes—namely, string beans, soybeans, and peas—a noticeable yellowing of the leaves was observed when borax was added at the rate of 0.75 pound, and in these cases a reduction in stand took place. The wheat plants grown at Arlington Farm on the plot fertilized with manure treated with from 2 to 3 pounds of borax to each 8 bushels, as noted on page 883, were yellow during the first 3 or 4 months of growth. When the growth started in the spring, however, the plants became green, and the yield of the grain was 90 per cent of the

control yield, more than that obtained from the unmanured control plot. The yellowing of the leaves is an unmistakable sign of injury, although in some cases the plant can recover, or at least is not sufficiently injured to cause a reduction in the yield.

Haselhoff (7) states that the action of boron is more marked on beans than on oats or corn, and that it can be seen when small amounts of boron are present in the soil and when no action injurious to plant growth is evident. He says further that small amounts of boron stimulate the growth of beans and corn, while large amounts produce injury. In his experiments beans absorbed boron in proportion to the amount present in the soil up to a certain limit. The plants examined by Haselhoff contained from 0.04 to 0.17 per cent of boron, which is more than was found in these experiments, with the exception of the plants grown at Orlando, Fla. (Table VII). He suggests that for safety the amount of boron in the soil be less than 0.0001 per cent. According to Brenchley (5), peas are stimulated by relatively high concentrations of boric acid, but with larger applications of boric acid the toxic action was well marked on the leaves, which tend to become brown and to die in a characteristic manner.

There is some evidence in the literature to indicate that small amounts of boron stimulate plant growth. Brenchley (5) states that below a certain dilution boron tends to produce stronger roots and shoots. Large amounts of boron are known to be toxic to practically all plants, with the exception of certain fungi.

In these experiments, where in most cases more boron was added than was necessary to act as a larvicide, no stimulating action was noted. On the contrary, an injurious action was seen with leguminous plants, which became yellow and did not show a good stand. Tomatoes, beets, lettuce, potatoes, radishes, corn, oats, and wheat appeared normal when grown in the presence of amounts of boron which produced injury to leguminous plants. When borax is added to manure at the rate of 0.62 pound to each 8 bushels and the manure is applied to the soil at the rate of 15 tons per acre, 0.0011 per cent of boron is added to the soil. This quantity of boron may injure leguminous plants, but did not injure the other plants tested, although no stimulation was noted. If the borax-treated manure is mixed with untreated manure, as would be done in many cases, since it is necessary to treat manure with borax to destroy fly larvæ during only a portion of the year, it is possible that the percentage of boron would be sufficiently reduced to bring about a stimulating action on plant growth.

In connection with the stimulating action of boron, it may be mentioned that nitrites and nitrates were detected in three or four borax-treated manure piles at New Orleans (6, p. 19), while the corresponding control piles contained no nitrites or nitrates, and several soils fertilized with borax manure have shown more nitrates than the check soils. A

stimulating action of boron on the nitrifying bacteria seems to follow in certain cases.

The results at Orlando, where the same amounts of boron were added to the soil as at other points, but where the toxic action of the boron was marked and where soluble boron was found in the soils after several months, indicates that many factors are involved in the absorption of boron and its effect on plants, and that definite conclusions in studies of this nature should be drawn with great care. These results are submitted as a preliminary study of this question. It is our purpose to test the cumulative action of boron in soils.

SUMMARY

(1) It apparently made little difference in the quantity of boron absorbed by the plants tested whether boron was added to the soil as borax or as calcined colemanite. The addition of lime with borax had no definite effect in preventing the absorption of boron. Wheat and oats absorbed very little boron, while leguminous and succulent plants absorbed comparatively large amounts.

(2) Wheat, beets, cowpeas, and tomatoes grown in pots in the greenhouses contained boron principally in the tops of the plants, and, with the exception of the beets, comparatively little or none in the roots.

(3) The fruit of the tomato plants contained only traces of boron, while the fruit of the cowpea contained large quantities. Lettuce grown in the greenhouse absorbed boron in proportion to the amounts present in the soil.

(4) Potatoes grown in the open showed, when mature, a small amount of boron in the tops and relatively large amounts in the roots and tubers.

(5) The leguminous plants, string beans, soybeans, and cowpeas, which were very sensitive to boron, showed when grown in plot tests a more equal distribution of the boron among the roots, tops, and fruit than the other plants tested.

(6) Radishes grown in plots contained much larger quantities of boron in the tops than in the roots. Analyses of entire plants of wheat, corn, peas, and oats grown on plots in the South showed the absorption of boron in all cases, the peas absorbing the most. All of the control plants contained at least a trace of boron.

(7) Samples of soil from some of the control plots showed the presence of acid-soluble boron, while several similar samples of soil from certain boron-treated plots showed no acid-soluble boron. Usually more soluble boron was found in the treated soil than in the control soil.

(8) The yield of wheat from a plot heavily treated with borax was 90 per cent of the manured-control yield and greater than the yield from the unmanured control. The wheat grains were sound and contained but a trace of boron.

(9) The yield of tomatoes in pot tests was unaffected when borax was added in amounts to produce 0.0018 per cent of boron in the soil, but when the amount was increased to 0.0030 per cent, a reduced yield resulted.

(10) Numerous factors influence the absorption, distribution, and action of boron in plants.

(11) No more than 0.62 pound of borax or 0.75 pound of calcined colemanite should be added to each 10 cubic feet of manure, and when using the boron-treated manure in growing leguminous plants, the manure should be mixed with untreated manure before being applied to the soil. For other plants, boron-treated manure should not be used at a higher rate than 15 tons per acre.

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FURTHER STUDIES ON PEANUT LEAFSPOT

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INTRODUCTION [1]

A report of investigations of certain fungous diseases of peanuts has previously [2] been made. Since the appearance of that report the investigations have been continued for the purpose of obtaining additional data on certain phases of the work. Opportunity had not been afforded prior to the present year to test under field conditions the efficacy of rotation and seed treatment in the control of leafspot, *Cercospora personata* (B. and C.) Ellis. Definite experimental data upon the agencies concerned in the distribution of leafspot had not been secured; neither had an effort been made to definitely correlate the destructiveness of the disease with the presence of certain climatic conditions. It was the primary purpose of the present work to secure information upon these phases of the subject. The results of these studies are, therefore, recorded as additions to the information contained in the previous publication [3] upon investigations which were begun four year ago under the Adams fund.

ROTATION TESTS FOR LEAFSPOT CONTROL

Because of the fact that the leafspot organism was found to live on fallen leaves in the field from one season to the next,[4] it was recommended as a rational method of control that the same fields be not planted to peanuts in successive years. Observations on the effectiveness of rotation were made at several widely separated points in the State, with the representative results which are recorded in Table I.

In many cases it has been difficult to get reliable information as to the crops previously grown upon the fields in which these studies were made, since the tenants knew nothing of the system of cropping employed prior to their tenure. In determining the percentage of plants affected, the

[1] The writer received valuable aid in the field tests from R. C. Lett, farm adviser for Tuscaloosa County, Ala., on whose farm the seed-treatment tests were conducted, and from S. A. Wingard, who carefully and arduously assisted in the field studies. Indebtedness is hereby acknowledged to both gentlemen for these several services.

[2] Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 127–150, 5 pl. 1914. Bibliography, p. 148–149.

[3] Wolf, F. A. Op. cit.

[4] When these leaves [diseased leaves which had remained out of doors from November until May] were kept moist as when placed in moist chambers, conidia were abjointed. Additional evidence that the fungus remains viable is to be found in the fact that leaf spots developed during May, on young plants, in a field which had grown a badly diseased crop the previous season. (Wolf, F. A. Op. cit., p. 135.)

total number of plants on a certain area was first counted, and then a count was made of those plants which were diseased. A plant having only a single spot on one of its leaves was regarded as diseased. Several attempts were made to determine the decrease in yield due to leafspot, but no satisfactory method has been found and the figures given are only approximate, since they were obtained by determining the average difference in the number of peas borne on 10 healthy and 10 diseased bunches having apparently the same-sized tops. It will be noted that the percentage of diseased plants in fields designated as 1 to 7, which are representative of rotations, varies from 13.5 to 100 per cent. When the results for the fields numbered 4 and 8 are contrasted, the former having borne no peanuts previously for 11 years and the latter having grown four successive crops, with 95 and 100 per cent, respectively, of the plants diseased, with practically no difference in the severity of attack, one is forced to conclude that rotation in itself is not to be regarded as a control measure against peanut leafspot. These results came somewhat as a surprise to the writer. Several reasons for the inefficacy of rotation as a means of leafspot control will be brought out later in this report. It might be suggested at this point, however, that this much overworked and overrecommended suggestion for the control of plant maladies is not a panacea, but requires experimental proof for each particular trouble for which it is recommended.

TABLE I.—*Summary of rotation tests with peanuts made in Alabama in 1915*

Field No.	Location.	Previous crops on soil.	Date of examination.	Plants affected.	Decrease in yield of peas.
				Per cent.	*Per cent.*
1	Auburn...	Peanuts had been grown 2 years before.....	Sept. 6	100	(a)
2	Eutaw....	Peanuts had not been grown for several years previously.	Aug. 28	54	5
3	...do.....	Peanuts had not been grown for 4 or 5 years previously.	30	41	4.5
4	...do.....	No peanuts had been planted for at least 11 years....	31	95	19.5
5	...do.....	No peanuts had been planted the previous year; no previous record available.	Sept. 1	26	(a)
6	. do.....	No peanuts in field the previous year.....	1	100	(a)
7	...do.....	No peanuts in field for 4 years previously.....	1	13.5	(b)
8	...do.....	Peanuts had been grown during each of the 4 preceding years.	Aug. 27	100	20

[a] Not estimated. [b] Negligible.

SEED DISINFECTION FOR LEAFSPOT CONTROL

Seed treatment for the control of leafspot was recommended in a previous report [1] for two reasons. It had been found that conidia adhere to the surface of the shells, and it had been noted repeatedly that the disease occurs in fields not previously planted to peanuts. It was suggested that solutions of copper sulphate or formaldehyde be used in dis-

[1] Wolf, F. A. Op. cit., p. 134. "The prevalence of leaf spot in lands not previously cultivated is not uncommon . . . conidia and conidiophores have been found in the centrifuged washings of peas."

infecting. In case the former was employed, the peas were to be immersed for 15 minutes in a solution containing 1 pound of copper sulphate to 20 gallons of water; in case the latter was used, 1 pint of formaldehyde to 20 gallons of water, the peas to be steeped for an hour. Tests of the effectiveness of these seed treatments were made during the past season (1915) at Eutaw, Ala. One field, designated as field 10, had previously grown several successive crops of peanuts; the other, field 11, had not been cropped with peanuts at least during the four preceding years. Each field was divided into four plots. Plot 1 in each field was planted with unshelled peanuts which had been immersed in copper sulphate; those in plot 2 were not shelled and were immersed in formaldehyde; those in plot 3 were given no treatment; in plot 4 no fungicide was employed, and the peanuts were shelled prior to planting. The conditions in field 10, as noted in three successive examinations, are given in Table II.

TABLE II.—*Summary of results of leafspot tests on field 10 in 1915, infested with leafspot*

Plot and treatment.	Aug. 6.	Aug. 14.	Aug. 21.
Plot 1. Peanuts, not shelled, steeped in copper sulphate:			
Total number of diseased leaves on 25 plants	882	1,492	3,201
Number of diseased leaves per plant—			
Maximum	79	110	273
Minimum	10	11	23
Plot 2. Peanuts, not shelled, steeped in formaldehyde:			
Total number of diseased leaves on 25 plants	755	1,437	3,020
Number of diseased leaves per plant—			
Maximum	63	144	241
Minimum	3	5	29
Plot 3. Peanuts, not shelled, no treatment:			
Total number of diseased leaves on 25 plants	1,022	2,028	4,234
Number of diseased leaves per plant—			
Maximum	94	178	297
Minimum	7	12	39
Plot 4. Peanuts, shelled, no treatment:			
Total number of diseased leaves on 25 plants	545	1,184	2,749
Number of diseased leaves per plant—			
Maximum	45	128	204
Minimum	2	15	26

Infections were observed in this field as early as July 26 and were probably present at a much earlier date. Plot 3 will be seen to have had a larger number of diseased leaves on August 6, and during the two successive weeks, than did any of the other plots. It would naturally follow from this that seed disinfection is not without appreciable effect. It was felt, however, that it would be necessary to duplicate these results in several localities during several seasons before one could safely conclude that seed treatment is of any practical value, especially in the light of the data to be subsequently presented.

Field 11, which can be directly contrasted with field 10, really shows the result of seed treatment coupled with rotation. No tabulation for field 11, such as has been made for field 10, has been prepared, but the important facts in regard to this field are as follows: Leafspot was not apparent until August 6, 11 days after it was first seen in field 10. Only five plants in the whole field were found to be affected on this date, and

the disease was evidenced by only one or two spots on each leaf. Two of these plants were found in plot 1, one in plot 2, and two in plot 4. It will be noted that on this date plot 1 of field 10 had a maximum of 79 affected leaves on a single plant, plot 2 had 30, plot 3 had 94, and plot 4 had 45. A final count of the number of diseased leaves in field 11 was made on September 1, with the result that 12 per cent of the leaves in plot 1 were affected, 11 per cent in plot 2, 15 per cent in plot 3, and 14 per cent in plot 4. It should be said in explanation that none of these plants had more than six affected leaves, and most of them had only one or two, upon which there were at most only a few spots. Ten days prior to this a final count on field 10 showed a minimum of 23 diseased leaves per plant and a maximum of 297. This number would, no doubt, have been considerably greater by September 1.

The most significant conclusion that one is forced to make from these tests is that seed treatment, either by itself or in conjunction with rotation, does not eliminate peanut leafspot. This conclusion is further supported by the results obtained from the rotation tests given in Table I. The peas used in planting fields 1, 2, 5, 6, and 7 were shelled prior to planting, thus eliminating the danger of introducing infective material at the time of planting. In these fields, 100, 54, 26, 100, and 13.5 per cent, respectively, of the plants were affected with leafspot. The peas used in planting fields 3 and 4 were not shelled, and 41 and 95 per cent, respectively, of the plants were diseased. As can readily be seen from these figures, the removal of the shells prior to planting contributed nothing toward keeping the crop free from disease.

DAMAGE SUSTAINED BY PEANUT PLANTS AS A RESULT OF LEAFSPOT

In order to measure the degree to which leafspot affects the foliage of peanuts, an effort was made to determine the relation between the total leaf area and the diseased area of a peanut plant. The plant used was taken from field 10 and may be regarded as a plant having an average proportion of diseased tissues. The method employed consisted in weighing pieces of paper corresponding in area to the total and the diseased leaf surface. From paper of good quality, pieces, each equal in area to one of the leaves of the plant, were cut. After these had been weighed, areas corresponding to the diseased parts of the leaves were outlined, and these areas were then removed. The paper leaf areas with the excised diseased areas were again weighed with the following computed results: The total weight of the leaves on a single plant is found to be 64.07 gm. Of this weight, 20.10 gm. are wholly free from spots; 12.39 gm. are dead as a result of the attacks of *Cercospora personata* and have for the most part fallen off; the remainder, 31.58 gm., are regarded as diseased leaves. Of these diseased leaves 10.18 gm., or 32.04 per cent, are occupied by the fungus. When 12.39 gm. and 10.12 gm. are combined, it is found that 35.07 per cent of the entire leaf area is lost to photosynthetic activity. It

is realized, of course, that these figures represent only an approximation, because the method itself is inexact. It is believed, however, that the approximated losses in yields of from 5 to 20 per cent given in Table I are reasonable, when one considers that there has been a loss to the plant of about 35 per cent in its active leaf area.

TESTS ON DISSEMINATION OF LEAFSPOT BY AIR AND WIND

Previous work on air currents as an agency in the dispersal of the leafspot fungus yielded only negative results.[1] It was believed, however, in spite of this negative evidence, that conidia are carried short distances by the wind.

The purpose of the tests herein reported was not only to determine whether or not the wind acts as an agent in dissemination of the conidia of *Cercospora personata*, but also to ascertain the conditions of temperature and humidity which might influence its maximum or minimum prevalence in the air. The tests were conducted at Eutaw and Auburn, Ala. The tests at Eutaw, Ala., at which place 210 exposures of plates were made, covered the entire period, nights as well as days, from August 9 to August 26, with the exception of August 15 and August 22. The tests at Auburn, Ala., were conducted from September 6 to September 11 and were made to substantiate the tests made at Eutaw, Ala.

The method formerly employed consisted in the exposure for varying lengths of time of sterile agar in Petri dishes. This method is open to objection for the reasons that at certain times the conidia of *Cercospora personata* germinate poorly or not at all and the development of colonies proceeds so slowly that they are likely to be obscured by more rapidly developing forms. It was decided, therefore, to use essentially the method employed by Burrill and Barrett [2] in their study of the dispersal of *Diplodia zeae*. Stations 2, 4, 6, and 8 feet distant from the nearest peanut plant were established. A frame to hold the exposure plates in a vertical position about 8 inches from the ground was made. This frame could be moved at the beginning of each exposure, to permit the plates to face toward the prevailing wind. Glass plates 4 by 5 inches were smeared with glycerin only on the side directed toward the peanut plants. Four sets of exposures of three hours duration each were made during the period from 6 a. m. to 6 p. m. One set of exposures of 12 hours duration was made nightly from 6 p. m. to 6 a. m. Rains interfered somewhat with this routine. Plates exposed during a rain were washed off, and those exposed in the periods following rain were found to be free from conidia. Readings of the temperature and relative humidity were made at the beginning of each set of exposures. The

[1] "All attempts to gain definite data showing that the wind is a carrier of the conidia have thus far been unsuccessful." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 134. 1914.)

[2] Burrill, T. J., and Barrett, J. T. Ear rots of corn. Ill. Agr. Exp. Sta. Bul. 133, p. 63–109, 11 pl. 1909.

exposed plates were brought into the laboratory as soon as possible after collection, were placed edgewise in a glass funnel, and the glycerin and contents washed off into a vial with a 2 c. c. pipetteful of 95 per cent alcohol. The stream of alcohol used in washing the plates was permitted to play slowly along the upper edge. The washings were then permitted to evaporate until only a few drops remained in the vials. By examination with the low-power lens of a microscope the number of conidia in these few drops could then be determined.

This method is open to two serious objections. Many of the spores were not washed from the plate by this method, as evidenced by a test in which a plate washed according to the method described and found to have entrapped three conidia of *Cercospora personata* was afterwards washed, using a wash bottle as a means of driving a stream of 95 per cent alcohol forcibly against it, and was found to have nine additional conidia. The other objection, which was encountered by Heald, Gardner, and Studhalter,[1] consists in the fact that it is practically impossible to spread a film of glycerin uniformly on a glass slide and have it remain so for three hours. The results shown in Table III are therefore not representative of the number of conidia that were actually entrapped, but convincingly prove that the conidia of *C. personata* are wind borne.

TABLE III.—*Results of tests of glycerin plates exposed to air currents at Eutaw, Ala.*

Date of exposure.	Number of plates exposed.		Number of plates with adhering conidia.		Rainfall.	Maximum number of conidia of *Cercospora personata* on any plate.	Total number of conidia entrapped during the entire period.	
	Day.	Night.	Day.	Night.			Day.	Night.
					Inches.			
Aug. 9	12	3	5	1	0	4	15	2
10	12	3	4	0	1.58	3	10	0
11	12	3	5	0	0	4	11	0
12	12	3	5	1	0.43	4	15	2
13	12	3	10	0	0	4	24	0
14	12	3	8	1	0.18	4	16	2
16	12	3	2	0	2.00	2	3	0
17	12	3	2	0	0.37	1	2	0
18	12	3	6	1	0	3	9	1
19	12	3	5	0	0.04	4	12	0
20	8	2	2	0	0.13	3	4	0
21	8	2	4	0	0	3	7	0
23	8	2	3	0	0.02	2	5	0
24	8	2	3	1	1.12	3	6	1
25	8	2	3	1	0	3	5	1
26	8	2	5	0	0	2	6	0
Total	168	42	72	6	.....	.....	150	9
Total day and night	210		78		.....	.....	159	

It is not deemed necessary to give a detailed daily record of the actual routine pursued. It will be seen that only 78 of the 210 plates exposed

[1] Heald, F. D., Gardner, M. W., and Studhalter, R. A. Air and wind dissemination of ascospores of the chestnut-blight fungus. *In* Jour. Agr. Research, v. 3, no. 6, p. 493–526, pl. 63–65. 1915. Literature cited, p. 525–526.

were found to have adhering conidia. The usual number found was three or four on each plate. The occurrence of rain and heavy dews will in part account for the relatively small number of plates upon which conidia were found. Rain fell on 9 of the 16 days during which these tests were made. The plates washed off by these rains numbered 26. Three sets of exposures of three plates each remained free from conidia in the periods immediately following rain. In many cases one plate only of each set gave positive evidence in the period following. Only six out of the 42 plates exposed at night yielded any positive results, owing principally to the occurrence of dews.

At no time during the period in which these tests were made, as will be seen, was there a maximum period of spore dispersal. Conidia were present in the air, except where it had been rendered free from them by precipitation, during the entire period. This is in accord with the increase in amount of leafspot shown in the successive counts made in field 10 and recorded in Table II. There was approximately twice as much leafspot in field 10 on August 14 as on August 6, and twice as much on August 21 as on August 14. No correlation between these increases and the temperature and humidity records could be discovered, and these figures have consequently been omitted from Table III. The idea formerly entertained[1] that the occurrence of peanut leafspot is correlated with certain moisture and temperature conditions is now regarded as without foundation. Such a correlation would be meaningless in view of the positive evidence, next to be reported, that insects act as carriers of leafspot. Details of the tests conducted at Auburn, Ala., are not tabulated, since the work accords with the work done at Eutaw, Ala., and substantiates the significant fact that air currents are agents in the dissemination of *Cercospora personata*.

INSECTS AS AGENTS IN DISSEMINATION OF THE LEAFSPOT ORGANISM

The fact that the leafspot fungus is air-borne explains in part at least the failure to secure perfect control in the tests in which rotation and seed treatment were combined. No tests have been made, however, upon the distance which the conidia may be transported by the wind. The most distant exposures were only 8 feet from the nearest diseased plant. It seems unlikely that air dispersal could account for severe infection in fields in which both rotation and seed treatment had been practiced and which were from ¼ to ½ mile distant from the nearest infected field. It was therefore suspected that certain insects, among which grasshoppers are the most important, are agents in this spread of leafspot.

[1] "Apparently infection with Cercospora is in some manner correlated with certain moisture and temperature conditions. . . The ravages of *Cercospora personata* seem to attain their maximum severity after a dry period followed by excessively sultry weather. . ." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 133. 1914.)

A relatively meager literature dealing with the subject of insects as carriers of fungi producing plant diseases has accumulated. Since the most important publications upon this subject are summarized in a recent excellent paper by Studhalter and Ruggles,[1] an historical review is purposely omitted at this time. These authors find that certain insects belonging to the orders Hemiptera, Coleoptera, Diptera, and Hymenoptera are carriers of the chestnut-blight organism. Because of the positive evidence secured in the few studies previously made on insects as agencies in the dissemination of plant diseases, it will not be surprising if it is found in future investigations that insects are a very important factor in the dispersal of many plant-pathogenic fungi.

The insects used in these tests were collected in diseased peanut fields near Eutaw, Thomasville, Marion Junction, Greensboro, and Auburn, Ala., and placed in sterile test tubes or flasks plugged with cotton. After being brought into the laboratory, each insect was dropped into a measured amount of water, in case it was desired to determine the number of conidia upon its body. After agitating the tubes vigorously a drop of the wash water was examined under the low-power lens of a microscope, the number of conidia in the drop were counted, and from this the total number of conidia was estimated.

In case fecal discharges were examined, each deposit was macerated in a drop of water on an object slide, and a count was made with the aid of the low-power lens. Because of the presence of undigested bits of plant tissue and the impossibility of one's being sure that no conidia escaped notice and that none were unwittingly counted twice, these determinations can not be exact. They very closely approximate the true number, however, since several counts of the same slide were made and the average taken as the final number.

A total of 75 insects collected in five different counties has been examined in the course of these tests, 54 of which gave positive results. Four orders of insects—namely, Orthoptera, Lepidoptera, Coleoptera, and Hemiptera—were represented among the positive tests. Of the 56 grasshoppers and katydids examined, 38 were found to be bearers of *Cercospora personata*. No attempt has been made to classify these Orthoptera, but several different genera were represented. Of the roasting-ear worms, *Heliothis obsoleta*, which were examined, nine were found to void conidia of Cercospora in their feces. Eight members of the Coleoptera were examined, six of which gave positive results. Three of these were lady beetles, *Megilla maculata;* one a blister beetle, *Epicauta vittata;* and the other two were fireflies, *Chauliognathus* sp. A single member of the Hemiptera, one of the leaf hoppers, was examined and found to be a carrier.

[1] Studhalter, R. A., and Ruggles, A. G. Insects as carriers of the chestnut blight fungus. Penn. Dept. Forestry Bul. 12, 33 p., 4 pl. 1915.

Table IV records the results of an examination of 36 of the 75 insects collected. The remainder of the record is not given, since it would add nothing which is not indicated in this tabulated portion.

TABLE IV.—*Record of examination of insects for conidia of Cercospora personata*

No.	Name of insect.	Date of collection.	Locality.	Number of conidia of *Cercospora personata*.		Other fungi.	Remarks.
				On body.	In feces.		
1	Grasshopper.....	Aug. 10	Eutaw, Ala.	1	8	.....	
2	.....do.....	13	.....do.....	1	.....	.....	No note was made of the occurrence of *Cercospora personata* or other organism in feces.
3	Roasting-ear worm (*Heliothis obsoleta*).	14	.....do.....	2	.....	.....	
4	Grasshopper.....	16	.....do.....	4	.....	.....	
5	.....do.....	16	.....do.....	1	.....	.....	
6	.....do.....	16	.....do.....	5	.....	.....	
7	.....do.....	26	.....do.....	4	.....	.....	
8	.....do.....	26	.....do.....	6	.....	.....	
9	.....do.....	26	.....do.....	3	.....	.....	
10	Firefly (*Chauliognathus* sp).	26	.....do.....	3	.....	.....	
11	Grasshopper.....	Sept. 6	Auburn, Ala.	.....	1,250	Species of Alternaria, Helminthosporium, and Fusarium.	Seven insects were placed in a flask and within a half hour after their capture they were examined. By agitating them in 25 c. c. of water conidia from the surface of the bodies and from the feces are included.
12	.....do.....	7	.....do.....	0	0	2,500 conidia of *Helminthosporium Ravenelii*.	
13	.....do.....	8	.....do.....	10	8	Fusarium, Alternaria.	
14	.....do.....	8	.....do.....	0	13	Species of Fusarium and Alternaria.	
15	.....do.....	8	.....do.....	0	0	.....	
16	.....do.....	9	.....do.....	0	18	.....	No determination of conidia on bodies was made.
17	.....do.....	9	.....do.....	0	250	.....	
18	.....do.....	9	.....do.....	0	200	.....	Single fecal discharge.
19	Roasting-ear worm (*Heliothis obsoleta*.)	9	.....do.....	0	1,050	.....	
20	Lady beetle (*Megilla maculata*).	9	.....do.....	8	0	.....	
21	Roasting-ear worm (*Heliothis obsoleta*).	10	.....do.....	0	50	.....	
22	.....do.....	10	.....do.....	0	17	.....	
23	.....do.....	10	.....do.....	0	39	.....	Total number of conidia contained in three fecal discharges. The presence of conidia upon the bodies was not determined.
24	.....do.....	10	.....do.....	0	27	.....	
25	.....do.....	10	.....do.....	0	0	.....	
26	.....do.....	10	.....do.....	0	17	.....	
27	.....do.....	10	.....do.....	0	28	.....	
28	.....do.....	10	.....do.....	0	25	.....	
29	Blister beetle (*Epicauta vittata*.	10	.....do.....	0	27	.....	Two fecal discharges were examined.

TABLE IV.—*Record of examination of insects for conidia of Cercospora personata*—Continued

No.	Name of insect.	Rate of collection.	Locality.	Number of conidia of *Cercospora personata*.		Other fungi.	Remarks.
				On body.	In feces.		
30	Lady beetle (*Megilla maculata*).	Sept. 10	Auburn, Ala.	9	0	.....	
31	Grasshopper.....	18	.....do.....	0	6	Several hundred conidia of *Helminthosporium Ravenelii* present.	
32	Katydid.....	18	.....do.....	0	92	.....	Two discharges.
33	Grasshopper.....	18	.....do.....	0	3	Many *Fusarium* sp. conidia.	
34	.....do.....	20	.....do.....	0	6	Few *Alternaria* sp. conidia.	
35	.....do.....	20	.....do.....	0	0	*Puccinia cassipes* B. and C., *Helminthosporium Ravenelii*.	Over 500 spores of each estimated to be present in a single discharge.
36	Leaf hopper.....	20	.....do.....	8	0	.....	

Grasshoppers were found to carry *Cercospora personata* conidia on their bodies and also to void them in their feces. The number of conidia to be found within and upon any individual insect depends naturally upon whether or not it has eaten diseased tissue within a short time prior to its capture. The largest number of conidia of *C. personata* found in a single fecal discharge of a grasshopper brought in from the field was 250.

In order to ascertain whether or not feeding grasshoppers either avoid or select diseased leaf tissue, 13 were brought into the laboratory, where they could be closely observed and given diseased peanut leaves as food. Three of them seemed to prefer leafspot tissue, since they ate little except the affected tissue. The others were indifferent in their choice of food, but seemed not to avoid the diseased spots. The conidia in the discharges of some of these insects were too numerous to count.

Passage through the alimentary canal of grasshoppers does not destroy the power of germination of the conidia of *Cercospora personata*. Conidia which had been voided were found to germinate within 12 to 18 hours when placed in drops of water. In fact, some were found to have already germinated at the time of discharge. When it is realized that these conidia-laden discharges are suitable situations for spore germination and a favorable pabulum for subsequent growth, and that they are commonly deposited upon leaves, it is seen that this is not an impossible means of causing infection. Since grasshoppers, which have notoriously strong powers of flight, were among the insects examined with positive results, they no doubt are potent agencies in the dissemination of leafspot for considerable distances. It is believed that the peculiar results in the tests on rotation and seed disinfection, as well as the correlation between the presence of leafspot and certain temperature and moisture conditions

previously reported, is due in part to the fact that grasshoppers and certain other insects are carriers of the leafspot organism.

It might be interesting to note in this connection that it seems to be generally true that peanut fields in which grass and weeds had been permitted to grow unmolested, as exemplified by fields 4 and 6 in Table I, and which consequently afforded a more attractive feeding ground for grasshoppers, are much more severely attacked by Cercospora than those in which good cultivation had been given. Several small fields have also been found upon which chickens and turkeys ranged in which leafspot was doing inappreciable harm, while fields somewhat farther away from the farm buildings were seriously affected. It is believed that the relative freedom from leafspot here observed is to be attributed largely to the destruction of the insects by fowls.

In most cases no attempt was made to determine the presence of other fungi upon the insects taken. Among the other forms noted, however, were *Helminthosporium Ravenelii* B. and C., an organism very abundant upon the inflorescence of *Sporobolus indicus; Puccinia cassipes* B. and C., which is parasitic on species of Ipomoea, a common weed; and species of Alternaria and Fusarium. According to an estimate made, a single fecal deposit of a grasshopper contained 2,500 conidia of *Helminthosporium Ravenelii.* A katydid taken at Marion Junction and one at Auburn each voided a vast number of morning-glory rust spores. Insects 21 to 28 (Table IV) indicate the manner in which this form may carry infections for short distances. The blister beetle is another insect which feeds upon peanut plants and which therefore discharges conidia from its alimentary canal. The other species of insects taken appear to carry conidia only upon their bodies. It seems very probable, judging from the evidence at hand, that any insect which feeds upon peanut foliage is a disseminator of leafspot, and that any of them which frequent peanut fields may serve as carriers.

SUMMARY

(1) Rotation by itself is not effective under field conditions in eliminating leafspot, as evidenced by a field in which peanuts had not been grown for 11 years and in which 95 per cent of the plants were diseased by August 31, with an estimated loss in yield of 19.5 per cent.

(2) Seed disinfection with copper sulphate or formaldehyde before planting does not prevent leafspot. Shelling peanuts before planting to eliminate the danger of infection from conidia which may have been adhering to the surface of the shell does not prevent the disease. Seed treated in these ways, when planted on land which had previously borne diseased peanuts, produced a crop which was 100 per cent diseased. Seed treated and planted on soil which had borne no peanuts for at least four years previously produced a crop 13 per cent of whose plants were more or less affected with leafspot. Crop rotation, therefore, when combined with seed treatment, will not eliminate leafspot.

(3) An approximation of the total leafspot area involved by *Cercospora personata* showed that the photosynthetic area had been decreased 35.07 per cent. Estimations of decrease in yield of peas of from 5 to 20 per cent as the result of leafspot are therefore regarded as reasonable.

(4) No correlation between the presence of certain conditions of temperature and moisture and the prevalence of leafspot exists, because of the fact that air currents and certain insects are carriers of *Cercospora personata.*

(5) As the result of 210 glycerin exposure-plate tests at Eutaw, Ala., substantiated by a series at Auburn, Ala., it is concluded that *Cercospora personata* is wind borne. Seventy-eight of these 210 exposure plates gave positive results. At no time from August 9 to August 26 was there a period of maximum spore dispersal as revealed by the exposure plates. The maximum number of conidia entrapped on any single plate was four. This does not represent the true condition, since the method used in washing the plates failed to remove all conidia. Rains rendered the air temporarily free from Cercospora, and dew prevented the dispersal of conidia at night and in the early morning.

(6) From an examination of 75 insects collected in five localities, of which 54 gave positive results, it is concluded that insects are disseminators of the leafspot fungus. Four orders of insects are included in these positive tests: Orthoptera, represented by grasshoppers and katydids; Lepidoptera, by larvæ of *Heliothis obsoleta;* Coleoptera, by lady beetles, blister beetles, and fireflies; and Hemiptera, by leaf hoppers. Grasshoppers, katydids, roasting-ear worms, and blister beetles eat diseased peanut foliage and void conidia in their fecal discharges. A single deposit from a grasshopper contained 250 conidia of *Cercospora personata.* Another specimen discharged 2,500 conidia of *Helminthosporium Ravenelii* in a single deposit. Grasshoppers may also carry conidia on the surface of their bodies. Leaf hoppers, lady beetles, and fireflies transport conidia on their bodies as a result of having come in contact with diseased leaves. A larva of *Heliothis obsoleta* voided a maximum of 1,050 conidia of *Cercospora personata.* Other fungi, among which are *Puccinia cassipes*, *Alternaria* sp., and *Fusarium* sp., were found in the fecal discharges of grasshoppers and katydids.

(7) Alimentation in insects does not destroy the viability of *Cercospora personata.*

(8) Grasshoppers, because of their powers of flight, are capable of carrying the leafspot organism considerable distances. The ineffectiveness of crop rotation combined with seed treatment to eliminate leafspot from peanut fields is very probably due to the fact that air currents and certain insects are agents in its dissemination.

RELATION BETWEEN THE PROPERTIES OF HARDNESS AND TOUGHNESS OF ROAD-BUILDING ROCK

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It has for some time past become increasingly evident to engineers interested in the testing of road materials that from the standpoint of the road builder some of the most important physical properties of rock are not independent, but are more or less definitely related to each other. In 1913, Mr. L. W. Page,[1] Director of the United States Office of Public Roads and Rural Engineering, called attention to some of these points, and suggested that, as the volume of data relating to the subject became greater, it might be possible to determine the dependent variable by reference to suitable curves showing the relative values of tests for thousands of individual cases, and thus dispense with one or more of the tests now in use. The large amount of additional data which have accumulated since that time makes it possible to take up the subject again, with a view to determining just what physical tests are necessary in order to judge properly the fitness of a rock for use in road construction.

It is now generally recognized that any stone, to be suitable for use in macadam construction, must possess to a certain degree, depending on circumstances such as character of traffic and method of construction, three distinct physical properties, which may be briefly defined as follows:

(1) Hardness, the resistance which a rock offers to the displacement of its surface particles by abrasion;

(2) Toughness, the resistance which a rock offers to fracture under impact;

(3) Binding power, the ability which the dust from the rock possesses, or develops by contact with water, of binding the larger rock fragments together.

Of these, the first two are of particular interest from the standpoint of the present discussion, and they may be very briefly described as follows:

The degree of hardness of a rock is determined by what is known as the Dorry method. It consists essentially of subjecting a cylinder, 25 mm. in diameter, of the material to be tested, to the abrasive action of crushed quartz sand fed upon a revolving steel disk, against which the test

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specimen rests. The end of the specimen is ground away in inverse ratio to its hardness, so that the hardness may be computed by determining the loss in weight after any given number of revolutions of the disk. The coefficient of hardness discussed later is obtained by subtracting one-third of the loss in weight in grams from 20, after 1,000 revolutions of the disk.

The degree of toughness is determined by the Page impact method. A cylinder 1 inch in diameter and 1 inch high, cut from the rock specimen, is subjected to the impact caused by the free fall of a 2-kgm. weight dropped from successively increasing heights until the energy of the blow is sufficient to fracture the test specimen. The test consists of a 1-cm. fall for the first blow, followed by falls increased by 1 cm. after each blow until failure occurs. The height from which the weight drops when failure takes place is used as a measure of the toughness of the material.

Since the establishment of the Road-Material Laboratory by the United States Government, upwards of 3,000 samples, representing every known variety of road-building rock, and obtained from every State in the Union, as well as from foreign countries, have been subjected to the tests outlined above. The results of these tests are plotted in graphic form in figure 1. The coefficients of hardness are plotted as abscissæ and the factors of toughness as ordinates. Each small circle represents the corresponding hardness and toughness of an individual rock sample. The large circles represent the average of all the coefficients of hardness for each value of toughness. Hardness values range from 0 to 20 and toughness values from 1 to 47.

A study of this curve brings out the following points:

(1) That the average toughness for all tests made is about 9.

(2) That the average hardness increases with toughness, and that the rate of increase becomes less as the toughness values become larger.

(3) That individual values of hardness vary through wide limits for low values of toughness, and that the variations from the average decrease uniformly with the increase in toughness up to a certain point, about 20, after which they remain constant with very little variation from the average.

(4) That, when any given value for toughness falls within certain limits, which define the suitability of the material for macadam-road construction under different traffic conditions, the corresponding value for hardness will fall within similar limits for hardness.

The first three facts are clearly indicated, but in order to substantiate the last deduction it will be necessary to define the limiting values of hardness and toughness which experience has shown should be applied when judging the fitness of stone for use in macadam construction under different traffic conditions. Such limiting values for toughness are shown on the curve in the ordinates at 4.5, 9.5, and 18.5, and the

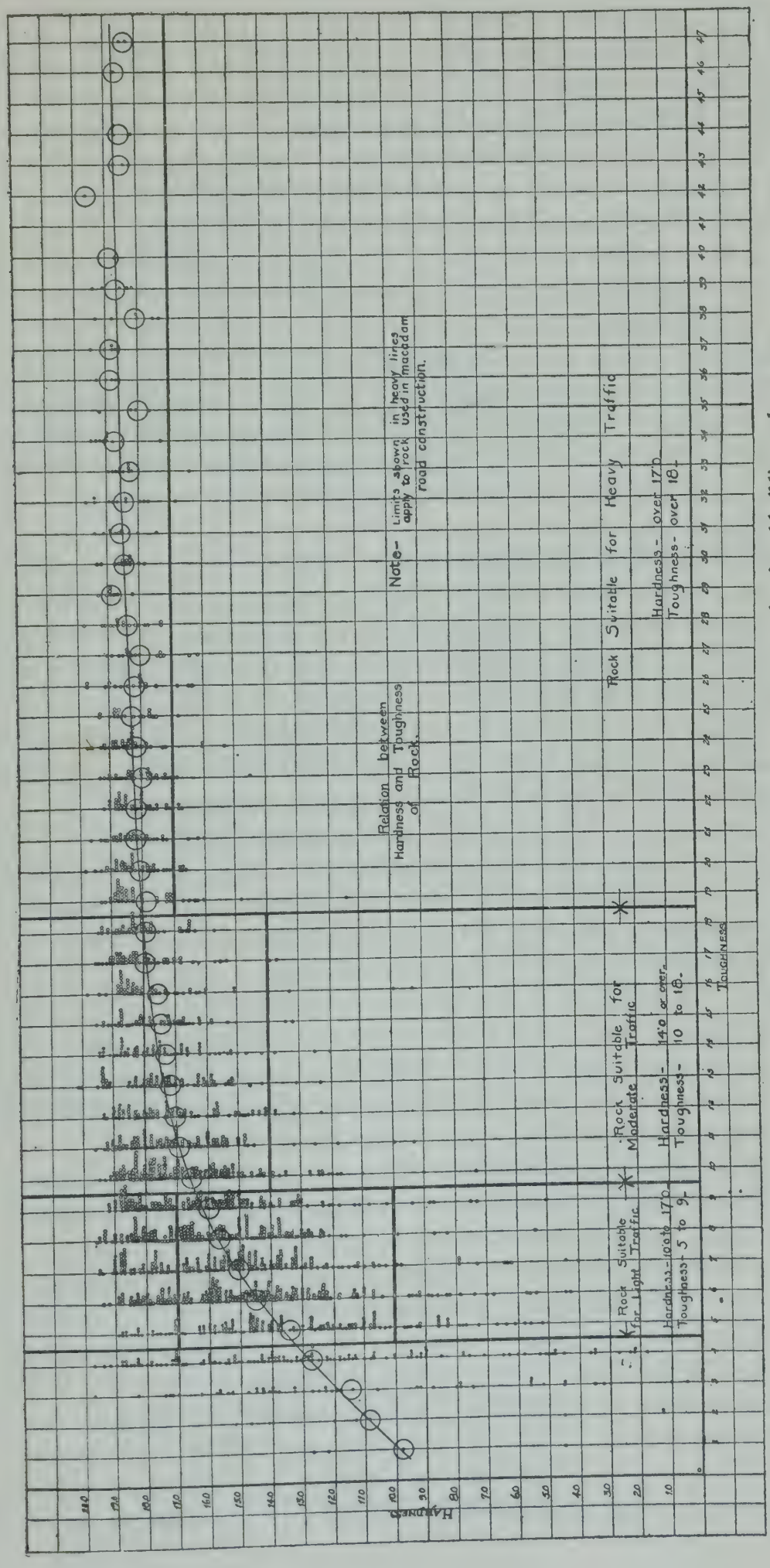
Relation between Hardness and Toughness of Rock.
Note- Limits shown in heavy lines apply to rock used in macadam road construction.
Rock Suitable for Heavy Traffic
Hardness - over 17.0
Toughness - over 18
Rock Suitable for Moderate Traffic
Hardness - 14.0 or over.
Toughness - 10 to 18.
Rock Suitable for Light Traffic
Hardness - 10.0 to 17.0
Toughness - 5 to 9
HARDNESS
TOUGHNESS

FIG. 1.—Curve showing the results of tests of about 3,000 samples of road-building rock.

corresponding limiting values for hardness at 10, 14, and 17. In other words, after making all allowances for variations due to local conditions, it may be fairly assumed that stone for use under light, horse-drawn, steel-tired vehicles should show a toughness of from 5 to 9 and a hardness of from 10 to 17; for moderate traffic a toughness of from 10 to 18 and a hardness of over 14, and for heavy traffic a toughness of 19 or over and a hardness of 17 or over. The terms "light," "moderate," and "heavy" in this connection refer to the total volume of traffic upon the road, calling, say, under 100 teams a day "light," 100 to 250 "moderate," and over 250 "heavy."

Practically all the values of hardness shown in figure 1 are above the various lower limits set by the best water-bound macadam-road practice.

For light-traffic conditions, 94 per cent of all the samples tested have a hardness of more than 10; for moderate traffic, 95 per cent have a hardness of more than 14; and for heavy traffic, 94 per cent have a hardness of more than 17.

In other words, if it be assumed that the curve (fig. 1) represents a fair average of all available types of road-building rock, it would seem that a determination of the toughness of any particular sample of rock shows, for all practical purposes at least, whether it is hard enough to be satisfactorily used in construction.

If the curve be referred to again, it will be seen that a large number of hardness tests appear above the upper limit of 17 set for light-traffic conditions. Although on its face this would indicate that a determination of the hardness is necessary in this instance, reference to test records show that by far the greatest number of these tests (about 75 per cent) are on granites, quartzites, and hard sandstones, which are unsuited for use in the wearing course of water-bound macadam roads, owing to their lack of binding power, as shown by actual test.

Finally, the results of 2,500 individual routine tests made by the Office of Public Roads and Rural Engineering show that for practical routine work the hardness test adds nothing to our knowledge of the value of any particular rock sample for use in water-bound macadam-road construction over that obtained from the toughness test.

While the binding or cementing value of a rock is a most important consideration from the standpoint of ordinary macadam construction, the same is not true of broken-stone roads which are surface treated or constructed with an adhesive bituminous material. The hardness of the rock is also of relatively less importance, owing to the fact that the fine mineral particles produced by the abrasion of traffic combine or should combine with the bituminous material to form a mastic which is held in place and protects the underlying rock from abrasion so long as by proper maintenance it is kept intact. The toughness of the rock, however, is of more importance, as the shock of impact is to a considerable extent transmitted through the seal coat and may cause the underlying fragments

to shatter. It would therefore seem that the minimum toughness of a rock for use in the construction of a bituminous broken-stone road or a broken-stone road with a bituminous-mat surface should for light traffic be no less than for ordinary macadam subjected to the same class of traffic. For moderate and heavy traffic, however, the same minimum toughness may probably prove sufficient, owing to the cushioning effect of the bituminous matrix. No maximum limit of toughness need, however, be considered for any traffic.

In the case of bituminous concrete roads, where the broken stone and bituminous material are mixed prior to laying and consolidation, it would perhaps appear advisable to set a minimum toughness of 6 or 7 for light-traffic roads instead of 5, in order to insure against the possibility of the fragments of rock which have been coated with bitumen being fractured under the roller during consolidation, and of 12 or 13 for moderate and heavy traffic, instead 10 and 19, as in the case of water-bound macadam roads.

For broken-stone roads which are to be maintained with dust palliatives, it would appear that the same limits of toughness should hold as for ordinary macadam.

For easy reference the following limits of toughness are given in Table I, as representing facts developed in the foregoing discussion. It is, of course, quite probable that these limits will require modification as the correlation of laboratory tests to service results becomes more perfect.

TABLE I.—*Limits for toughness for rock used in the construction of broken-stone roads*

Type of road.	Light traffic.		Moderate traffic.		Heavy traffic.	
	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.
Macadam	5	9	10	18	19	.....
Macadam with dust palliative						
Macadam with bituminous mat	5	.....	10	.....	10	.....
Bituminous broken stone with seal coat						
Bituminous concrete with or without seal coat	7	.....	13	.....	13	.....

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., FEBRUARY 14, 1916 NO. 20

NITROGEN CONTENT OF THE HUMUS OF ARID SOILS[1]

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HISTORICAL REVIEW

One of the most generally recognized characteristics of arid soils (16, p. 163; 15, p. 415; 17, p. 72; 13, p. 147)[2] is the high content of nitrogen contained in their humus, the *matière noire* of Grandeau (6, p. 148).

Attention was first called to this by Hilgard and Jaffa (10), who stated (p. 69):

> It thus appears that on the average the humus of the arid soils contains three times as much nitrogen as that of the humid; that in extreme cases the difference goes as high as 6 to 1.

It is somewhat remarkable that so few other investigators have made any attempt to test this generalization in the case of the soils from the arid portions of either this or any of the other continents.

Fulmer (5) determined the humus nitrogen in 53 soils from Washington, a State with winter rains and summer droughts. In the case of two soils from Skagit County, which has an annual precipitation of about 46 inches, he found 10.46 and 12.04 per cent, respectively, of nitrogen in the humus.

Nabokich (14, p. 339) reports six samples from Bessarabia with from 11.1 to 18.9 per cent of nitrogen in the humus. It seems probable, however, that he has confused the use of the term "humus" as employed on the continent of Europe (organic matter of the soil as determined by combustion with copper oxid) with the sense in which it is generally used in this country. However, he makes a direct comparison of the Danubian soils with those of California, as follows:

> In contrast with the soils of the dry steppes of southern Russia, the humus of the borders of the Danube is quite as rich in nitrogen as that of the soils of the steppes of California and Transcaucasia. The alluviums of the Danube are even richer than those of the Arax.[3]

[1] The work reported in this paper was carried out in 1911 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Assistant in Chemistry.

[2] Reference is made by number to "Literature cited," p. 915–916.

[3] Author's translation (14, p. 339).

Southern Bessarabia has an annual precipitation of about 15 inches, most of it falling during the growing season. Such a climate in this country is commonly referred to as "semiarid."

Loughridge (12, p. 87), in a comprehensive study of the distribution of humus in California soils to a depth of 12 feet, made nearly 1,000 determinations of humus nitrogen, stating that—

> of these there were about 64 where the humus was found to contain more than 10 per cent nitrogen, fourteen of these had from 15 to 20 per cent and but five had more than 20 per cent. . . The general average for all the soils, including the marsh lands, is 5.92 per cent for the first foot, 5.60 per cent for the upper three feet and 5.57 per cent for the entire depth of twelve feet.

Our work was an outgrowth of previous investigations in the same laboratory of the humus of semiarid soils. Samples from the semiarid prairies of Canada had been found by Alway and Trumbull (3) and Alway and Vail (4) to show percentages of nitrogen in the humus similar to those in soils from humid regions. In subsequent, as yet unpublished, studies by Alway and Trumbull and by ourselves many surface soils, representing various soil types and the different degrees of aridity found in Nebraska as well as many samples from the semiarid and desert portions of New Mexico and Arizona, were analyzed without finding even one in which the humus contained as much as 10 per cent of nitrogen. The question then naturally arose as to whether we would meet with similar results if we worked with arid soils from a region of winter rains and summer droughts. Having available a small collection of samples of California soils personally collected in 1909 by one of us in connection with another study, we subjected these to analysis. As our analyses were not fully confirmatory of Hilgard's conclusions, we delayed publication of the results, hoping to be able to continue the work with a more extensive series of samples from California. Since then, Loughridge (12) has reported his findings, with which ours are in general agreement. The question as to the conditions under which a high content of nitrogen in the humus is found in arid soils does not appear as yet at all satisfactorily answered. We present our data in the hope that some one more conveniently located for the collection of the necessary samples will take up the study.

The data upon which Hilgard's conclusions were based are given in the Annual Reports of the Agricultural Experiment Station of the University of California from 1884 to 1902. The method used for the determination of humus nitrogen is described by Hilgard (7, p. 247) and Jaffa (11, p. 35): "Two portions of 5 or 10 grams of air-dried soil (depending on richness in humus)" were placed in prepared filters, washed first with dilute (0.5 to 1.0 per cent) hydrochloric acid, until the filtrate gave no reaction for lime and magnesia, and then with distilled water to neutral reaction. Then the one portion was washed with repeated portions of 6 to 7 per cent ammonia solution until the washings became colorless while the other

was similarly treated with a 4 per cent potassium-hydroxid or a 3 per cent sodium-hydroxid solution. The ammonia solution was used for the determination of the humus, while in the other the humus nitrogen was determined by the Kjeldahl method. On the assumption that the same compounds had been dissolved by the two solvents, the percentage of nitrogen in the humus was calculated.

While Hilgard's conclusions were based upon determinations in which the humus was extracted with an alkaline hydroxid solution, he later suggested as an alternative the use of the ammonia solution (8, p. 22), this being concentrated and then mixed with magnesia and boiled before being subjected to the Kjeldahl determination.

The correctness of the assumption that the ammonia solution dissolves the same compounds or the same proportions of the total nitrogen as the alkaline hydroxids is open to serious question. A mere determination of the nitrogen removed by the two solvents does not suffice to decide the question. The ammonia is likely to combine with some of the dissolved organic matter of the soil, with the result that after the concentration of the extract, preliminary to the Kjeldahl digestion, there may still be present some nitrogen derived from the ammonia in addition to that extracted from the soil. The attempt to eliminate any such combined nitrogen by digestion with magnesia previous to the Kjeldahl determination is unsatisfactory, as the magnesia may decompose some of the nitrogen compounds extracted from the soil with the elimination of ammonia. A determination of the organic carbon in both solvents should be made, and if this is not the same the nitrogen in the alkaline hydroxid solution is not to be regarded as that corresponding to the whole of the organic matter dissolved by the ammonia.

EXPERIMENTAL WORK

We have confirmed Hilgard and Jaffa's (10) observation that after prolonged extraction of a soil with either ammonia or alkaline hydroxid solution the other fails to extract any appreciable amount of black material. Using 10-gm. portions of both a semiarid and a humid soil, we treated with a 4 per cent ammonia solution until the washings became colorless, placed the residues together with 500 c. c. of alkaline hydroxid solution in stoppered bottles, shook these at frequent intervals for eight hours, and then allowed them to stand overnight. In the case of potassium hydroxid, we tried concentrations of 64, 32, 16, 8, 4, 2 per cent and of sodium hydroxid of 36, 18, 9, 4.5, 2.25 per cent. In all cases the amount of coloring matter extracted was so small that the humus could not be satisfactorily determined even by the delicate photometric method (2). Accordingly, it seems safe to assume that the ammonia solution removes the dark coloring matter as completely as the alkaline hydroxids. However, there appears no reason for assuming that a definite relation exists between the quantity of this pigment and the

amount of ammonia-soluble matter in a soil. Comparisons of the color of the ammonia extracts with their content of dissolved matter show that this relation is variable for different depths in the same field and for the same depth in different localities (2, p. 13).

The large number of soils referred to above were analyzed, using the ammonia extract and magnesia, without finding any in which the humus contained as much as 10 per cent of nitrogen. A later critical study of the method showed that the results were not reliable, the amount of humus nitrogen found being affected by the extent to which the solution was concentrated before adding magnesia and also by the time of digestion with the latter. One result of this was that, while parallel determinations gave concordant results, those run one after the other, using the same ammonia solution, gave widely varying results.

The extraction of the humus by the Hilgard-Jaffa method (10) in the case of many soils, especially those of very fine texture, is extremely tedious, being in this respect similar to the Hilgard method for the determination of humus, for which in the case of some soils 10 days or even longer is necessary (7, p. 320). For this reason we sought to devise a more expeditious and convenient method. Using two representative soils, one a silt loam from the Nebraska Experiment Station farm containing 2.41 per cent of humus and 0.245 per cent of total nitrogen, and the other a clay loam from Indian Head, Saskatchewan, Canada, with 1.56 per cent of humus and 0.248 per cent of total nitrogen, we tried shaking 10 gm. of dry soil with 500 c. c. of a 4 per cent potassium-hydroxid solution for periods of 0.5, 1, 2.5, 5, 9, 12, and 24 days. During the working portion of the day the glass-stoppered bottles containing the mixtures were shaken at intervals of about one hour. With both soils the amount of nitrogen dissolved ceased to increase at the end of nine days. Repeated extraction of the same soil with fresh alkali solution, which might have given different results, was not tried.

This method was then compared with that of Hilgard and Jaffa (10), using in the case of five arid soils from California (Table I) both a 4 per cent potassium and a 6 per cent sodium-hydroxid solution.

TABLE I.—*Comparison of methods for the determination of humus nitrogen*

Determination.	Humus nitrogen.			Total nitrogen.
	New method.	Hilgard-Jaffa method.		
		With potassium hydroxid.	With sodium hydroxid.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
A. First	0. 162	0. 150	0. 176	
Second	. 159	. 152	. 180	
Third	.....	. 153	. 169	
Average	. 160	. 152	. 175	0. 260
D. First	. 023	. 021	. 025	
Second	. 020	. 020	. 018	
Third	.....	. 020	. 023	
Average	. 021	. 020	. 022	. 031
F. First	. 024	. 023	. 026	
Second	. 025	. 021	. 026	
Third	.....	. 020	.....	
Average	. 025	. 021	. 026	. 032
I. First	. 058	. 045	. 037	
Second	. 064	. 046	. 038	
Third	.....	. 049	. 041	
Average	. 061	. 047	. 039	. 104
L. First	. 047	. 034	. 030	
Second	. 047	. 035	. 035	
Third	.....	. 038	. 035	
Average	. 047	. 036	. 033	. 070

The results are only fairly concordant, but the extraction of nitrogen was as complete as by the Hilgard-Jaffa method, and for our study this was the most important consideration.

Using this method, employing a 4 per cent potassium-hydroxid solution and shaking at intervals for 9 days, we determined the humus nitrogen in 16 samples of arid soils from California (Table II). The humus was determined by the Hilgard method (1, p. 319). Duplicate and, in most cases, triplicate determinations were made of both the total nitrogen and the humus nitrogen, and duplicate determinations of the humus.

TABLE II.—*Relation of nitrogen to humus in arid soils from California*

Sample No.	Depth.	Location and description of soil.	Humus.	Humus ash.	Total nitrogen.	Humus nitrogen.	Nitrogen in humus.	
							Found.	Maximum possible.
	Inches.		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
A	0–3	Berkeley. Adobe, virgin.	1. 71	0. 46	0. 260	0. 160	9. 3	15. 2
B	0–3	.....do.....	1. 19	. 25	. 233	. 119	10. 0	19 6
C	0–6	Waterford. Alluvium, cultivated.	. 62	. 39	. 054	. 035	5. 6	8. 7
D	0–6	Ceres. Loam, cultivated.	. 31	. 25	. 031	. 021	6. 7	10. 0
E	0–3	Fresno. Red hog-wallow land, virgin.	. 47	. 52	. 026	. 019	4. 1	5. 5
F	0–6	Clovis. Red land, cultivated.	. 29	. 18	. 032	. 025	8. 3	11. 0
G	0–6	Fresno. Black adobe, cultivated.	1. 39	. 92	. 144	. 087	6. 3	10. 4
H	0–5	Fresno. Dry bog, virgin.	. 75	. 45	. 078	. 030	4. 0	10. 4
I	0–5	Fresno. Dry bog, cultivated.	. 84	. 29	. 104	. 061	7. 2	12. 4
J	0–5	Clovis. Red land, cultivated.	. 38	. 16	. 060	. 032	8. 8	15. 6
K	0–5	.....do.....	. 36	. 17	. 052	. 037	10. 4	14. 4
L	0–6	Delano. Alluvium, cultivated.	. 40	. 14	. 070	. 047	11. 8	17. 5
M	0–6	Delano. Red land, cultivated.	. 50	. 32	. 061	. 036	7. 5	12. 2
N	0–6	.....do.....	. 38	. 17	. 061	. 034	9. 0	15. 1
O	0–2	Delano. Red land, virgin.	1. 00	. 34	. 159	. 115	11. 5	15. 9
P	0–2	Delano. Alluvium, virgin.	1. 17	. 20	. 187	. 140	12. 0	16. 0
Average	.....	.....	. 73	.....	. 101	. 062	8. 3	13. 1

All the samples, except the two from Berkeley, Cal., were secured in the San Joaquin Valley in the vicinity of Modesto, Fresno, and Delano, where the normal annual precipitation amounts to 10.9, 9.0, and 6.1 inches, respectively. Samples A and B were both taken from the high hill just east of the buildings on the grounds of the University of California. Sample A is a composite of 20 samples from near the summit, and B of the same number from the lighter colored soil to the west, below the summit. Sample C was from a cultivated field east of Modesto and 4 miles west of Waterford. Sample D was from a fallowed field 3 miles south of Hickman and 12 miles east of Ceres, E from the virgin red hog-wallow land 7 miles north of Fresno, and F from the red lands 10 miles east of Clovis. The last-named had been under cultivation from 15 to 20 years. Sample G is a black adobe from the same farm as sample F, and the field had been under crop for about 7 years. Samples H and I are "dry-bog soils" from a hilltop near the farm from

which F and G were secured. H was from virgin soil, while I was from land which had been formerly cultivated, but allowed to revert to grass about 10 years before. Samples J and K were taken from two fallowed fields of red land about 2 miles east of Clovis. The remaining samples were from near Delano—L from a field under cultivation for 15 years and M and N from fallows on red land north of the White River.

Of the 16 samples only 5 show as high as 10 per cent of nitrogen in the humus. For the 6 samples of virgin soil the average is 8.5 per cent, with a maximum of 12.0 and a minimum of 4.0 per cent. For the 10 of cultivated soils the corresponding data are 8.1, 11.8, and 5.6 per cent, respectively. The maximum possible percentages of nitrogen in the humus—the relation of the total nitrogen to the humus—ranged from 5.5 to 19.6 per cent, with an average of 13.1. Hilgard (9, p. 424), in a comparison of the average composition of 313 arid and 466 humid soils, reports the former to show 0.75 per cent humus and 15.87 per cent of nitrogen and the latter 2.70 per cent of humus, with only 5.45 per cent of nitrogen.

There is no reason to doubt the reliability of the humus determinations upon which Hilgard's generalizations are based. A careful study (1) has shown that his method, as carried out by himself, gives results strictly comparable with those of the Moores-Hampton method. We have examined the original data on the humus determinations by Hilgard and his assistants and in only a very few cases do we find a humus-ash content sufficiently high to make the determination appear inaccurate. These percentages of humus ash, while not reported in the tables in Hilgard's articles discussing the relation of the nitrogen content of humus to climate, may be found in the original reports referred to above.

In that we found 5 out of 16 arid soils to have over 10 per cent of nitrogen in the humus after having failed to find any humid or semiarid soil with such a high percentage, our study tends to confirm the work of Hilgard that high percentages are to be found in the arid but not in the humid soils. This high nitrogen content of the humus, however, does not appear so general in the arid soils as to serve as an at all reliable means of identification.

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LIFE-HISTORY STUDIES OF THE COLORADO POTATO BEETLE

By PAULINE M. JOHNSON, *Scientific Assistant*, and ANITA M. BALLINGER, *formerly Preparator, Truck-Crop and Stored-Product Insect Investigations, Bureau of Entomology.*

INTRODUCTION

The experiments on the life history of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the details of which follow, were suggested by Dr. F. H. Chittenden, in charge of Truck-Crop and Stored-Product Insect Investigations of the Bureau of Entomology, and were conducted under his direction. These studies were necessarily carried on indoors for the most part and under somewhat unnatural conditions. Had they been conducted out of doors, the probabilities are that in any well-kept field of potatoes (*Solanum tuberosum*) the beetles would have passed through a period of estivation; and if the potatoes had been grown under weedy conditions, where the beetles had access to wild solanaceous plants, the third generation would have been produced. All experiments were performed in the District of Columbia during the season of 1914. The temperature during the period of the work was exceedingly high, with more than the normal rate of humidity.

GENERATION EXPERIMENTS

The overwintered beetles of this species made their first appearance after hibernation on April 29 on *Solanum jasminoides*, an ornamental plant growing in the insectary garden. Beetles were collected and pairs isolated in jars for experimental purposes. After feeding for a few days the females began depositing their characteristic orange-colored eggs (Pl. LXIII, fig. 1) in masses on the underside of the leaves near the tips. The egg masses averaged from 35 to 45 eggs each, except in two cases observed, in which as many as 70 and 72 eggs, respectively, were counted. When the potato plants first emerged from the ground, the beetles showed a decided preference for them, deserting the foliage of *S. jasminoides* for the more tender leaves of the potato.

The fecundity of single females, under the conditions described, is shown in Tables I to VIII.

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FIRST GENERATION

TABLE I.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female taken in copula on April 30, 1914, and placed in rearing jar with growing potato plant*[1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 4....	17	17	May 15...	24	24	May 25...	4	4
5....	0	0	16...	0	0	26...	0	0
6....	0	0	17...	0	0	27...	0	0
7....	0	0	18...	16	16	28...	0	0
8....	43	43	19...	0	0	29...	14	14
9....	0	0	20...	0	0	30...	16	16
10....	67	67	21...	10	10	31...	24	1,5,10,8
11....	31	31	22...	9	9	June 1...	3	3
12....	45	45	23...	0	0			
13....	28	28	24...	10	10	Total..	379	.....
14....	18	18						

[1] The male in this experiment died on June 10, the female on June 14.

TABLE II.—*Eggs produced by a single overwintered female of the Colorado potato beetle; pair collected at College Park, Md., and placed in rearing jar on May 11, 1914, with growing potato plant* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11..	78	32, 46	May 31...	70	21, 18, 31	June 19...	0	0
12..	51	31, 20	June 1...	25	25	20...	5	5
13..	31	31	2...	21	21	21...	0	0
14..	0	0	3...	0	0	22...	0	0
15..	0	0	4...	2	2	23...	9	9
16..	0	0	5...	0	0	24...	40	40
17..	90	36, 54	6...	0	0	25...	14	14
18..	0	0	7...	0	0	26...	0	0
19..	36	36	8...	0	0	27...	0	0
20..	32	32	9...	0	0	28...	0	0
21..	34	9, 11, 17	10...	6	0	29...	0	0
22..	0	0	11...	0	0	30...	0	0
23..	0	0	12...	0	0	July 1...	0	0
24..	34	34	13...	0	0	2...	0	0
25..	72	24, 48	14...	0	0	3...	0	0
26..	29	20, 9	15...	34	34	4...	0	0
27..	25	25	16...	0	0	5...	24	24
28..	47	47	17...	71	52, 19			
29..	33	33	18...	50	50	Total..	994	.....
30..	28	28						

[1] The male died on June 7, the female on July 7.

TABLE III.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female collected at College Park, Md., and placed in confinement on May 10, 1914, with growing potato plant* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	44	44	May 26...	0	0	June 9...	0	0
12...	16	16	27...	0	0	10...	0	0
13...	0	0	28...	0	0	11...	0	0
14...	0	0	29...	25	25	12...	0	0
15...	46	46	30...	0	0	13...	0	0
16...	0	0	31...	0	0	14...	23	23
17...	39	39	June 1...	23	23	15...	42	42
18...	0	0	2...	16	16	16...	33	33
19...	36	19, 17	3...	0	0	17...	0	0
20...	11	11	4...	0	0	18...	0	0
21...	0	0	5...	0	0	19...	0	0
22...	0	0	6...	0	0	20...	16	16
23...	0	0	7...	10	10			
24...	0	0	8...	0	0	Total.	389	.....
25...	0	0						

[1] The male died on June 23, the female on September 2.

TABLE IV.—*Eggs produced by a single overwintered female of the Colorado potato beetle; pair of adults taken in copulation and isolated in a rearing jar on May 11, 1914, with a growing potato plant* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	57	57	June 4...	56	20, 36	June 28...	0	0
12...	15	15	5...	41	41	29...	0	0
13...	15	15	6...	41	41	30...	33	33
14...	58	58	7...	45	45	July 1...	42	42
15...	0	0	8...	35	35	2...	0	0
16...	54	54	9...	60	60	3...	43	43
17...	57	57	10...	43	43	5...	35	35
18...	0	0	11...	57	57	6...	0	0
19...	33	33	12...	84	19, 65	7...	47	47
20...	25	25	13...	47	47	8...	61	22, 39
21...	21	21	14...	54	54	9...	13	13
22...	31	31	15...	55	55	10...	30	30
23...	34	34	16...	51	51	11...	0	0
24...	0	0	17...	39	39	12...	20	20
25...	56	56	18...	46	34, 12	13...	0	0
26...	26	26	19...	31	31	14...	0	0
27...	27	27	20...	0	0	15...	13	13
28...	0	0	21...	7	7	16...	8	8
29...	37	37	22...	1	1	17...	16	16
30...	42	42	23...	0	0	18...	0	0
31...	30	30	24...	0	0	19...	8	8
June 1...	25	25	25...	0	0	20...	14	14
2...	36	36	26...	0	0			
3...	0	0	27...	0	0	Total.	1,879	.....

[1] The male died on August 1, the female on August 20. In this experiment the duration of egg-laying extended over a period of 70 days, or 10 weeks.

TABLE V.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female in copula isolated on May 11, 1914, with growing potato in rearing jar* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 14..	36	36	June 3..	0	0	June 23..	6	6
15..	0	0	4..	39	39	24..	0	0
16..	43	43	5..	45	14, 31	25..	16	16
17..	0	0	6..	43	43	26..	0	0
18..	20	20	7..	35	35	27..	11	11
19..	0	0	8..	46	33, 13	28..	0	0
20..	0	0	9..	54	54	29..	38	38
21..	54	27, 27	10..	63	34, 11, 18	30..	0	0
22..	0	0	11..	62	30, 32	July 1..	0	0
23..	32	32	12..	24	24	2..	20	20
24..	33	33	13..	57	57	3..	8	8
25..	19	19	14..	34	34	4..	0	0
26..	25	25	15..	33	33	5..	0	0
27..	67	33, 34	16..	31	31	6..	0	0
28..	0	0	17..	34	34	7..	0	0
29..	40	40	18..	25	25	8..	4	4
30..	42	42	19..	0	0			
31..	48	12, 12, 24	20..	8	8	Total..	1, 301	.....
June 1..	32	32	21..	12	12			
2..	43	43	22..	19	19			

[1] The male died on June 25, the female on July 27. Temperatures: Maximum, 98° F.; minimum, 43° average, 72°.

Eggs which were deposited on May 4 hatched on May 12, and the larvæ (Pl. LXIII, fig. 2) fed ravenously until May 28, when they entered the ground to a depth of about 3 inches and transformed to pupæ on May 30. Adults emerged on June 9.

Eggs which were deposited on May 7 hatched on May 16. The larvæ became full grown, pupated on May 31, and entered the soil, from which the adults issued on June 10.

SECOND GENERATION

After the adults of the first generation had issued from the ground, three pairs were isolated while in copulation and placed in jars with potato leaves as food on June 17, 18, and 19, respectively.

TABLE VI.—*Record of egg deposition of first-generation female of pair 1 of the Colorado potato beetle, confined in rearing jar on June 17, 1914, and fed upon potato foliage* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
June 22..	22	22	July 5...	11	11	July 17...	47	47
23..	45	45	6...	0	0	18...	44	44
24..	11	11	7...	19	19	19...	45	45
25..	23	23	8...	24	24	20...	0	0
26..	0	0	9...	0	0	21...	0	0
27..	0	0	10...	0	0	22...	37	37
28..	44	44	11...	0	0	23...	0	0
29..	2	2	12...	0	0	24...	0	0
30..	15	15	13...	22	22	25...	0	0
July 1..	47	47	14...	0	0	26...	17	17
2..	27	27	15...	0	0	27...	11	11
3..	0	0	16...	0	0			
4..	0	0				Total..	513	.....

[1] The male in this experiment died on June 20, the female on August 4.

The male and female of pair 2, having been confined to the rearing jar on June 18, 1914, fed for a few days upon the potato foliage, after which they entered the ground for hibernation, the female depositing no eggs.

TABLE VII.—*Record of egg deposition of first-generation female of pair 3 of the Colorado potato beetle, confined in rearing jar on June 19, 1914, and fed upon potato foliage* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Nnmber of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
July 1...	4	4	July 10...	0	0	July 18...	38	38
2...	0	0	11...	0	0	19...	18	18
3...	32	32	12...	0	0	20...	38	38
4...	30	30	13...	0	0	21...	0	0
5...	38	38	14...	0	0	22...	15	15
6...	48	48	15...	0	0	23...	65	65
7...	46	46	16...	0	0			
8...	33	33	17...	59	22, 37	Total..	502	.....
9...	38	38						

[1] The male of this pair went into hibernation on July 20, the female on July 27. Temperatures: Maximum, 102° F.; minimum, 58°; average, 72°.

A mass of eggs which was deposited on June 30 by the female of pair 1 hatched on July 7. The larvæ became full-grown on July 23, pupated on July 25, and emerged as adults on July 31. Another mass of eggs laid on July 10 by the same female hatched on July 16, the larvæ pupating on August 5 and issuing as adults on August 11.

THIRD GENERATION

When the adults of the second generation had emerged, pairs were isolated as in previous experiments.

TABLE VIII.—*Record of egg deposition of second-generation female of a pair of the Colorado potato beetle, confined in rearing jar and fed upon potato foliage* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.
1914.		
August 20.....	19	19
21.....	48	48
22.....	0	0
23.....	45	45
Total.....	112	.....

[1] Temperatures: Maximum, 96° F.; minimum, 46°; average, 70°.

In the rearing experiments with the third generation the females of the second generation did not all oviposit. Four pairs began hibernation after feeding for several days. One mass of eggs deposited on August 4 hatched on August 9, the larvæ pupating on August 23 and the adults emerging on August 31. Another egg mass, which was deposited on August 21, hatched on August 26, and the larvæ, becoming full-grown on September 14, entered the ground for pupation, the adults emerging on September 23.

All of the beetles of this third generation were very active and fed voraciously on the foliage of the potato up to September 15.

LENGTH OF STAGES

Table IX shows the maximum and minimum number of days covered by each of the immature stages in each of the three generations, as obtained from the foregoing rearing experiments.

TABLE IX.—*Maximum and minimum length (in days) of immature stages of the Colorado potato beetle in each of the three generations*

Generation.	Egg stage.		Larval stage.		Pupal stage.		Total developmental period.	
	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.
First.....	7	9	15	18	10	10	30	37
Second.....	6	7	16	18	6	8	32	41
Third.....	5	5	14	19	8	9	27	35

NUMBER OF MOLTS AND DURATION OF INSTARS

Eggs of the Colorado potato beetle were segregated and watched carefully to determine the number of molts of the larvæ and the time spent in each instar. It was found that every larva has three molts, with an average of about three days for each instar. Tables X and XI show the dates and number of days required for the molts.

TABLE X.—*Number of molts and dates of molting of Colorado potato-beetle larvæ in 1914*

Experiment No.	Egg hatched.	First molt.	Second molt.	Third molt.
1	July 26	July 29	Aug. 2	Aug. 5
2	...do....	...do....	Aug. 1	Aug. 3
3	July 30	Aug. 2	Aug. 4	Aug. 8
4	...do....	...do....	...do....	Do.
5	...do....	...do....	...do....	Aug. 6
6	...do....	...do....	...do....	Aug. 8
7	Aug. 7	Aug. 9	Aug. 15	Aug. 19
8	...do....	Aug. 10	...do....	Do.

TABLE XI.—*Duration (in days) of instars of Colorado potato-beetle larvæ*

Experiment No.	First instar.	Second instar.	Third instar.
1	3	4	3
2	3	3	2
3	3	2	4
4	3	2	4
5	3	2	2
6	3	2	4
7	2	6	4
8	3	5	4
Maximum duration	.....	.....	2
Minimum duration	.....	.....	6

FALL MATING FOR SPRING EGG LAYING

The fact that the Colorado potato beetle may be observed mating in September in the latitude of the District of Columbia has probably given rise to the opinion that a third generation might be produced elsewhere—e.g., in Minnesota. This last generation, whether second or third, has been proved in one instance to be fertilized in the fall, the females on issuing being capable of depositing eggs in the spring without a second copulation. This was found to be the case with the generation which held over from 1914 and was observed in the spring of 1915, for a female came to the surface on March 8 and, without mating, deposited eggs on March 11 and 12, which hatched on March 20 and 21. These larvæ fed until March 30 and 31, when they pupated, the adults emerging on April 19,

1915. This was an indoor experiment, and the beetles had been kept in a warm room during this entire period. In the field the first adults were observed in the insectary garden May 4, 1915. It was quite cold during that period compared with the earlier season of 1914.

SUMMARY AND CONCLUSIONS

In the authors' experiments in 1914 in the District of Columbia eggs of the Colorado potato beetle were laid almost immediately after the first overwintering beetles were collected in copulation in the spring. These overwintering beetles fed continuously until September 7, when the last one died. The adults of the first generation upon emergence fed for a short time; some of them went into hibernation, but most of them laid eggs for a second generation. Likewise, some adults of the second generation hibernated, while others laid eggs from which adults of the third generation developed. Dr. Chittenden has stated [1] that in the course of his investigations he was not able to get the beetle to breed more than twice in a season without a period of estivation; but from the few eggs that were laid in the second generation the authors were able to rear the species through three generations without a resting period.

In 1908 Popenoe [2] made experiments with this insect in tidewater Virginia, and reared it through three generations, but all the beetles of the third generation died. In this experiment the heat was still greater than in Washington in 1914, and the insects were not isolated in large numbers and were not well fed, which accounts for the dying of the third generation.

The entire developmental period from egg to adult was passed, as previously stated by Dr. Chittenden, in approximately four weeks.

Particular attention is called to the fact that the female, far from laying the small number of eggs attributed to this species, is capable of laying, in one case under actual observation, 1,879, while a second female deposited 1,301 eggs. The former record exceeds any hitherto published, so far as known. It should be stated, however, that during 1913 Mr. W. O. Ellis,[3] of the Iowa Agricultural Experiment Station, obtained from a single female of the species a total of 1,686 eggs, and that Messrs. Girault and Zetek [4] took 1,578 eggs from a single beetle.

From the experiments reported herein it is evident that there are three completed generations of the Colorado potato beetle in the District

[1] Chittenden, F. H. The Colorado potato beetle (Leptinotarsa decemlineata Say). U. S. Dept. Agr. Bur. Ent. Circ. 87, p. 8–9. 1907.

[2] Popenoe, C. H. The Colorado potato beetle in Virginia in 1908. U. S. Dept. Agr. Bur. Ent. Bul. 82, pt. 1, 8 p., 2 pl. 1909.

[3] Ellis, W. O. Leptinotarsa decemlineata Say. *In* Jour. Econ. Ent., v. 8, no. 6, p. 520–521. 1915.

[4] Girault, A. A., and Zetek, James. Further biological notes on the Colorado potato beetle, Leptinotarsa 10-lineata (Say), including observations on the number of generations and length of the period of oviposition. II, Illinois. *In* Ann. Ent. Soc. Amer., v. 4, no. 1, p. 74. 1911.

of Columbia and localities having the same mean temperatures, part of the adults of the first and second generations hibernating, wnile the remainder lay eggs from which the second and third generations develop. Furthermore, the possibility of a partial fourth generation is suggested by the fact that the beetles of the third generation were active and feeding voraciously during September, 1914. This insect is to be found in all stages during the summer months, and there is much overlapping of generations.

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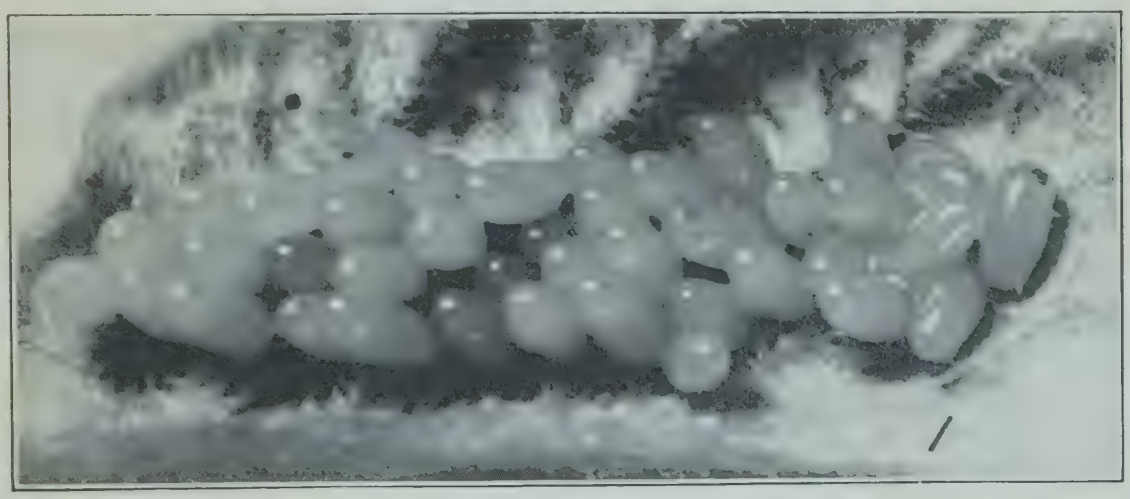
PLATE LXIII

Colorado potato beetle (*Leptinotarsa decemlineata*):

Fig. 1.—Egg mass, highly magnified. Original.
Fig. 2.—Young larva, highly magnified. Original.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SOME FACTORS INFLUENCING THE LONGEVITY OF SOIL MICRO-ORGANISMS SUBJECTED TO DESICCATION, WITH SPECIAL REFERENCE TO SOIL SOLUTION

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INTRODUCTION

The following outline is suggestive of the complexity of the problem of determining the relative influence of the various factors affecting the longevity of microbes subjected to desiccation:

(1) Properties of the organism which probably depend on species differences.
  (*a*) Spore formation.
  (*b*) Capsule formation.
  (*c*) Peculiarities of cell composition.
(2) Physiological differences in organisms resulting from treatment before drying.
  (*a*) Temperature of cultivation.
  (*b*) Nutrition.
  (*c*) Age of culture.
  (*d*) Virulence and other properties.
(3) Nature of the medium in which the organism is suspended before drying.
  (*a*) Its possible plasmolyzing effect.
  (*b*) Its content of protective or water-retaining substance.
(4) Physical structure of the substratum upon which drying occurs.
  (*a*) Smooth, nonabsorbent surfaces.
  (*b*) Textile fibers or fabrics.
  (*c*) Soil, etc.
(5) Effect of physical agencies.
  (*a*) Light.
  (*b*) Temperature.
  (*c*) Variations in humidity, etc.

Only a few of the points in this outline will be treated in detail in this paper.

HISTORICAL REVIEW

A review of the literature reveals only the facts that are usually incorporated in recent text books on microbiology. The longevity of spores is too well known to demand discussion at this time. It is recognized in the literature that the presence of a gelatinous capsule is an excellent means of protection against adverse circumstances, especially desiccation. It is also noted by Liesenberg and Zopf (13)[2] that with an organism

[1] This paper represents part of a piece of work planned and prepared for publication by the senior author, but executed almost entirely by the junior author as a part of the requirements for the degree of Master of Science.

[2] Reference is made by number to "Literature cited," p. 941–942.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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like *Streptococcus mesenterioides* the naked modification—i. e., the form developed on a medium containing no sugar and having no capsule—succumbs more quickly as a result of desiccation than does the encapsulated form. *S. mesenterioides* (13, p. 244) has been found to resist desiccation for a much longer period if developed on a saccharin medium than on one which contains no sugar. Revis (20) shows that two types of colon organisms which developed a mucilaginous type of growth were the ones which survived longest in soil. In another article (21) he suggests that the slime formed by organisms of the colon type may add to the water-absorbing and water-retaining capacity of the soil, and may therefore promote the longevity of that organism. Löhnis (15) says that not only the spores but also the bacteria with slimy walls endure the effects of desiccation very well. Lafar (13) emphasizes the importance of making a distinction between organisms like *S. mesenterioides*, which surrounds itself with a gelatinous envelope, and organisms which carry on a slimy fermentation—i. e., conversion of sugar outside the cell into mucinous matter—without themselves being inclosed in capsules. Jensen (11, p. 323) uses the terms capsule formation and slimy fermentation interchangeably and regards the process as protecting the organism against desiccation.

Buchanan (2, p. 378) offers a very comprehensive review of the literature on the nature and morphological origin of bacterial slimes. Some describe gum formation as the result of a true fermentation of carbohydrates by bacteria, calling it an extracellular synthesis, others calling it a true synthetic process, but not necessarily due to an extracellular ferment. Most of the bacterial gums reported in the literature are described as carbohydrates of the formula $(C_6H_{10}O_5)_n$. Bacterial slimes classed as dextrans are described by Bräutigam, Kramer, Ritsert, Scheibler, and many others (2). Lipman, Greig-Smith, Maassen, and Laxa (2) found levulan to be the specific gum of several slime-forming bacteria. Schmidt-Mühlheim, Hueppe, Emmerling, Greig-Smith, Laurent, Ward, and Seiler (2) describe bacterial gums having the characteristics of galactans. A few nitrogenous bacterial gums are mentioned, but they appear to be less common than those of a carbohydrate nature. The protective action of these gums has been ascribed to their water-retaining capacity.

Exclusive of organisms with such special protective structures as spores or capsules, it appears to be true that certain species are more resistant than others. Neisser (4) found that the organisms of typhoid fever and diphtheria were the most resistant; cholera, influenza, bubonic plague, and gonococci the least; and the pus-forming cocci, meningococcus, and tubercle bacillus of intermediate resistance. Briscoe (1) credits the tubercle bacillus with a greater resistance than most non-spore-bearing organisms. This power of resistance is no doubt due in part to the waxy or fatty substance found largely in the outer layer of the tubercle bacillus.

Ficker (8) states that the temperature at which the organisms are cultivated and their ability to resist drying at different temperatures stand in a certain relation. Drying at a higher temperature does not always produce a more rapid effect and the drying at a lower temperature a more gradual effect. He concluded that cultivation at a temperature below the optimum produces an individual with the greatest resistance to desiccation. His results (7) with the drying of cholera vibrio cultures of different ages indicate that cultures 1 or 2 days old endure desiccation better than older cultures, but of these two the 48-hour culture is less sensitive to drying at 37° C. than is the 24-hour culture. The results of Kitasato and Berckholtz, quoted in the same article, show about the same resistance in cultures from 1 to 5 days old. Cultures older than these showed a marked decrease in resistance, due not only to the fact that there were fewer living organisms present in the same mass of an old culture, but these surviving organisms possessed in themselves less vitality than did the vibrios from younger cultures. Ficker (7) also demonstrated in the case of the cholera vibrio that a virulent strain was more resistant than an avirulent strain. Ficker's experiments (8) showed that transfers of old cholera vibrios from the surface of agar to distilled water resulted in a disturbance of the turgor of the cell which was so injurious as to make its death, when desiccated, occur much sooner than was the case when they were suspended in physiological salt solution and dried. With young cultures the reverse was true. Suspension in tap water or distilled water appeared to have the same effect, but desiccation after suspension in physiological salt solution was quickly injurious. He explains this on the basis that since the drying process resulted in an increase of concentration of the salt solution, the cell was subjected to both plasmolysis and desiccation. The explanation is not complete, however, for a broth of the same salt content as the physiological salt solution was favorable to both young and old cultures. He found (8) the cholera vibrio to retain its vitality longer when dried from a suspension in milk or broth than in distilled water, tap water, physiological salt solution, serum, or saliva. Ficker (8) also showed that a greater longevity resulted after drying on cover-glass films when the organisms were first cultivated on a solid medium and then suspended in fresh broth or milk, than when they were grown in those liquids and then dried on cover-glass films prepared directly from the medium in which they developed.

Peiser (17) showed that the thermal death point of lactic-acid bacteria when determined in milk is higher than when determined in bouillon. Numerous examples are cited of the long preservation of organisms in a dry state when surrounded by nitrogenous or albuminous material. Chester (4) says that *Pseudomonas radicicola*, when dried in thin films on glass, perishes very rapidly, but that it may live 11 to 16 days on cotton. Harding and Prucha (23) have shown that *Bacterium campestris* may

live for as long as 13 months on cabbage seeds, but when dried on cover slips it is dead at the end of 10 days. Briscoe (1) says that this difference is no doubt largely due to the difference in the hygroscopic moisture retained by these substances. He found that tubercle bacilli lived only 8 to 12 days when dried in thin smears on glazed-paper slips. *Bacillus coli*, *B. violaceus*, and *B. prodigiosus*, according to his experiments, were even more sensitive dried under those conditions.

As to the relative merits of desiccation in room air and in a desiccator, some fairly positive statements have been obtained. Chapin (3, p. 195) says that as a rule bacteria live longer when dried in a desiccator than when dried in the open air under natural conditions. Ficker (7) showed that the rapid drying of organisms in a desiccator over calcium chlorid or sulphuric acid was preferable to drying in ordinary room air. Ficker's experiment (7), in which the organisms were placed alternately in a desiccator and a moist chamber for a couple of hours at a time, resulted in the organisms so treated dying much more rapidly than did those which were left in the desiccator continuously for the same length of time. Löhnis (15) states that frequent changes between drying and remoistening are most injurious, but that rapid drying in a space with a "rarefied atmosphere" (in a desiccator) is comparatively favorable. Unpublished experiments of J. Simon have shown that the repeated drying and moistening of the soil is much more detrimental to nodule bacteria than keeping the soil constantly dry. Chester (4), in his experiments with *P. radicicola*, found that an important condition for the successful preservation of the organism in a dry state was to keep the culture sealed from the air and in a dark, cool place.

The evidence obtainable from the literature in regard to the length of time an organism may live in air-dry soil and the factors responsible for its longevity are neither definite nor complete. Lipman (14, pp. 228 and 230) says that—

> Under air-dry conditions each soil grain is surrounded by a very thin film of moisture designated as hygroscopic water . . . According to Hall the film of hygroscopic moisture is about 0.75μ (0.00003 in.) thick . . . Nevertheless, it will be seen that the moisture, even in air-dry material, is deep enough to allow the bacteria a reasonable amount of protection. This will account for the survival of non-spore-bearing bacteria in dry soil for a long time. Indeed, instances are on record of the isolation of *Azotobacter* and *Nitrosomonas* from soils that had been kept in the laboratory for several years.

Löhnis (15, p. 67) says that—

> vegetative cells can better endure drying when they are in soil. With spores also this is true. The resistance of spores dried in earth is usually found to be higher than that of spores dried on cotton, silk, glass, etc.

Duggar and Prucha (6) found that after the rapid drying out of soil cultures there remained a large number of living organisms whose vitality

would extend over a considerable period. Nestler (16) investigated an old herbarium and found that even after 23 years 90,000 colonies could be obtained from 1 gram of soil. Azotobacter (12) remain alive in soil samples if these samples are kept for 160 days in a desiccator and then 148 days in an air-tight condition. Germano's (9) results seemed to indicate that the organisms of typhoid fever and diphtheria did not live as long in soil as on fabrics, although the diphtheria bacillus averaged 20 to 40 days' longevity in all trials in soil. Firth and Horrocks (3) found that the typhoid bacillus would live for 23 days in dry sand. Pfuhl (18) found the typhoid bacillus to live 28 days in dry sand and 88 days in moist garden earth. The bacillus of dysentery, on which he experimented at the same time, lived only 12 days in sand and 101 days in moist garden earth. Briscoe (1) found the tubercle bacillus to live 213 days in garden soil.

But little work has been done to determine the effect of different soil types on the longevity of organisms dried in them. The data offered in the literature on this point are not only scanty but far from recent. Modern texts hold that dust does not offer protection to many pathogenic organisms, the dangers due to ordinary dust being much exaggerated according to Rosenau (22, p. 72) and Chapin (3, p. 263). Dempster (5) found that the cholera vibrio lived only a short time in perfectly dry soil, but survived for a prolonged period in soil containing a small amount of moisture. The typhoid bacillus showed a greater tenacity of life in soil than did the cholera vibrio, but entire desiccation proved to be quickly fatal to it also. Comparison of the longevity of these organisms in white sand, gray sand, garden mold, and peat showed that with the exception of peat, which apparently contained substances toxic to the organisms, the nature of the soil did not have a direct influence on them. The vitality of the organisms appeared to depend rather on the moisture content of the soil than on its composition. Our experiments on the longevity of soil organisms in different types of soil have led to a modified conclusion. The longevity of vegetative cells in air-dry soil is probably, as Lipman (14, p. 228) suggests, due mainly to the presence of moisture in the hygroscopic form, although undoubtedly the presence of organic colloidal substances with a tendency to retain moisture and with other properties is of importance. Van Suchtelen, in speaking of the analysis of soil solution as quoted by Giltner (10, p. 154), makes certain statements, which, on account of their immediate bearing on this subject, deserve direct quotation. He says:

> In many cases there was found in the soil solution a slime. This must be regarded as the first experimental proof of the presence of this substance in the soil, and it is not impossible that much of the irregular behavior of the life in soil can be explained to some extent with a knowledge of this slime. If I may be permitted, I should like to call your attention to the possibility of this substance having an effect on desiccation, diffusion, and other processes.

It is the above statement which has stimulated and formed the basis of the experimental work recorded herein. No progress has been made in the direction of an explanation of the nature of this slime. Its effect on the prolongation of the life of micro-organisms subjected to desiccation has been the object in view.

EXPERIMENTAL STUDY

An experiment was conducted to determine whether an organism may receive protection from the solution in which it is suspended before being subjected to desiccation in sand. For this work were used cultures of *P. radicicola* grown for five days at room temperature on nitrogen-free ash agar. For suspension the following solutions were employed:

(1) Physiological salt solution.
(2) Physiological salt solution + 0.1 per cent of agar.
(3) Physiological salt solution + 0.1 per cent of gelatin.
(4) Physiological salt solution + 0.1 per cent of albumin.
(5) Physiological salt solution + 0.1 per cent of gum arabic.
(6) Physiological salt solution + 0.1 per cent of soluble starch.

With the exception of the albumin solution these were all prepared by dissolving 1 gm. of the dry substance in a small amount of salt solution and then making it up to a volume of 1,000 c. c. They were found to be practically neutral to phenolphthalein. On account of the difficulty of dissolving powdered egg albumin it was found necessary to use raw white of egg, a quantity being taken which by computation contained 1 gm. of albumin. As albuminous solutions may be heated to 100° without coagulation if slightly alkaline, this solution before sterilization was made −10° F. S. by the addition of N/1 sodium hydroxid. After sterilization (which with all six was accomplished by the Tyndall method, 30 minutes heating in flowing steam on four successive days) the N/1 sodium hydroxid was neutralized with N/2 hydrochloric acid, leaving the albumin solution like the other five, practically neutral.

Suspension of the bacterial growth from four agar slopes was made in 250 c. c. of each of the above solutions. For the purpose of securing initial counts 1 c. c. of each suspension was diluted and plated on nitrogen-free ash agar. Twelve flasks of quartz sand were then inoculated from each of the six solutions, 5 c. c. to a flask. The sand had been prepared after the method described by Rahn (19). It was heated with diluted hydrochloric acid, washed several times, first with tap water and then with distilled water, heated on a water bath until almost air dry, and then heated at least 30 minutes over a free flame. Fifty gm. of the dry sand was placed in 100 c. c. Erlenmeyer flasks, which were plugged with cotton. Sterilization was accomplished by heating for 45 minutes in the autoclave under 15 pounds' pressure.

The inoculated flasks were kept in a dark, well-ventilated place at a temperature of 22° to 25° C. At intervals the number of organisms per

gram of sand was determined by the plate method, samples being taken from two flasks representing each suspension solution. Nitrogen-free ash agar was used for all plates and these were kept 10 days at a temperature of 22° to 25° C. before counting.

It is evident from Table I that the counts are irregular and not such as to form a basis for any positive conclusions. This is due in part to the fact that the fluctuations in numbers from time to time were so extreme that it was difficult to determine what dilutions should be used to obtain plates from which accurate counts might be made. One great mistake in this trial was the addition to the sand of a quantity of moisture which was sufficient to permit the multiplication of the organisms for three weeks after inoculation of the flasks. In later trials the addition of less moisture lessened the period of multiplication. The bacteria were not actually subjected to desiccation until after January 27, by which time the difference in the numbers of organisms developing on the five different substances was such that a fair comparison of their water-retaining capacity during the process of drying was not possible. Although it is true that after a desiccation period extending over almost four weeks (from the last of January to February 24) there were greater numbers of living organisms in the flasks to which the albumin solution had been added, it is possible that this would not have occurred had not the organisms in those flasks reached enormous numbers just previous to the period of drying, because of the superior nutritive qualities of this substance.

TABLE I.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gum-arabic solution.	Starch solution.	Gelatin solution.	Albumin solution.
Jan. 2[a]	60,000	60,000	60,000	60,000	60,000	60,000
7	27,400	428,700	30,000	60,500	626,400	−10,000
15	1,711,000	3,651,000	63,160	2,143,000	3,974,000	(?)
27	674,800	328,000	60,000	468,100	1,335,600	3,677,000
Feb. 13	1,000	1,000	−1,000	−1,000	10,000	30,000
24	−50	−50	50	50	−50	200

[a] Initial counts.

Another experiment of the same nature was made with the following solutions:

(1) Physiological salt solution.
(2) Physiological salt solution + 0.1 per cent of agar.
(3) Physiological salt solution + 0.1 per cent of gelatin.
(4) Physiological salt solution + 0.1 per cent of gum arabic.
(5) Nutrient broth.
(6) Milk.
(7) Soil solution (extracted from garden soil, sandy loam, by the method of Van Suchtelen).[1]

[1] All soil solutions were furnished by Mr. J. Frank Morgan, Research Assistant in Bacteriology.

The bacterial growth from one agar slope was suspended in 12 c. c. of each of the above solutions, and 1 c. c. was diluted and plated quantitatively on nitrogen-free ash agar. From each of the seven suspensions 2 c. c. was added to each of five flasks of quartz sand, which was of the same quality and prepared exactly as in the preceding trial.

These flasks were kept in a dark place at 22° to 25° C. Quantitative determinations, made at intervals, are based on plates from but a single sample of each set, consequently the opportunity for error is materially increased. It can not, therefore, be claimed that these figures (Table II) show accurate comparisons. However, it is quite evident that between March 26 and April 17, during which time the sand was so dry as to make the multiplication of organisms impossible, the rate of decrease in the numbers of organisms taken from broth, milk, and soil solution was noticeably less than that of organisms from the other solutions. This implies a certain protection gained from the presence of nitrogenous or albuminous constituents in the milk or broth. To what substance or substances in the soil solution such protection should be credited can not be stated definitely. The slime, mentioned by Van Suchtelen (10, p. 154), may be of influence in this connection.

TABLE II.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Broth.	Milk.	Soil solution.
March 18..	1, 100, 000	1, 500, 000	1, 440, 000	1, 613, 000	1, 024, 000	1, 176, 800	1, 460, 000
26..	−10, 000	−10, 000	10, 125, 000	−10, 000	19, 967, 000	185, 000	40, 000
April 6....	−25	25	50	−25	220, 000	405, 000	8, 600
17....	−25	−25	−25	−25	−25	−25	−25

An additional experiment was conducted employing the same cultures used in the previous experiments. The procedure was the same, except that as a basis for quantitative determinations two samples were taken from each set instead of one. As the plates from several of the flasks showed no colonies whatever on May 3, even in the lowest dilutions, which represented 1/25 gm., it was thought advisable in making the next determinations, on May 13, to take 1-gm. samples from these flasks and mix them directly with the melted medium in the Petri dish instead of plating 1 c. c. of a dilute suspension as previously done. It is quite evident that the direct mixture of the sand with the plating medium tends to give higher counts than those secured by plating the washings of the sand, for in the latter case a large number of organisms undoubtedly remain attached to the sand particles instead of being washed off into the suspension. This difference in technic may account for the apparent increase in numbers in certain cases, as shown by the last plating.

TABLE III.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Albumin solution.	Broth.	Milk.	Soil solution.
April 16	1,648,000	2,144,000	1,901,000	3,234,000	360,000	1,477,000	4,026,000	1,266,000
May 3	−25	25	−25	−25	56	428,625	515	391
13	.....	.....	116	.....	2	432,000	106	3,080

The figures in Table III offer little except a general confirmation of the results of the two other experiments. As the sand was air dry after April 26, it may be understood that the counts on May 3 and May 13 represent the numbers surviving 7 and 17 days desiccation, respectively. Attention must be called to the fact that the lack of figures to show the comparison in increase of bacteria in the different solutions between April 16 and April 26 makes it impossible to overlook entirely the function of these different solutions in their nutritive capacity. Plates were made on April 26, but the nitrogen-free agar made up with maltose instead of saccharose proved an unfortunate choice; for no colonies whatever developed, although, as seen by the two subsequent platings, living organisms were then present in abundance. However, the favorable influence of the soil solution, whether it may be as a food material for soil organisms or a protection during desiccation, can not be disputed.

An experiment was conducted to compare the longevity of *P. radicicola* dried in quartz sand and in clay-loam garden soil. As in the foregoing experiments, the organism was grown for five days at room temperature on nitrogen-free ash agar. The bacterial growth from one agar slant was transferred to 12 c. c. of physiological salt solution and the mixture shaken thoroughly, and 1 c. c. of the suspension was diluted and plated quantitatively. To the two flasks each of clay loam and quartz sand were added 2 c. c. of the bacterial suspension. The clay loam had been sifted and air dried. The quartz sand had been prepared after Rahn's method, described previously. Fifty-gm. portions of each were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and sterilized by heating in the autoclave for 45 minutes under 15 pounds' pressure.

The inoculated flasks were shaken to distribute the organisms throughout the sand or soil, and then kept in a dark, well-ventilated place at a temperature of 22° to 25° C. The number of living organisms per gram of sand and loam was determined at intervals by plating quantitatively from two samples of each.

TABLE IV.—*Difference in longevity of Pseudomonas radicicola dried in quartz sand and in clay-loam soil*

Date.	Sand.	Clay loam.
April 16	1,648,000	1,648,000
May 3	25	42,133
13	.....	33,025

The bacterial growth from one agar slope was suspended in 12 c. c. of each of the above solutions, and 1 c. c. was diluted and plated quantitatively on nitrogen-free ash agar. From each of the seven suspensions 2 c. c. was added to each of five flasks of quartz sand, which was of the same quality and prepared exactly as in the preceding trial.

These flasks were kept in a dark place at 22° to 25° C. Quantitative determinations, made at intervals, are based on plates from but a single sample of each set, consequently the opportunity for error is materially increased. It can not, therefore, be claimed that these figures (Table II) show accurate comparisons. However, it is quite evident that between March 26 and April 17, during which time the sand was so dry as to make the multiplication of organisms impossible, the rate of decrease in the numbers of organisms taken from broth, milk, and soil solution was noticeably less than that of organisms from the other solutions. This implies a certain protection gained from the presence of nitrogenous or albuminous constituents in the milk or broth. To what substance or substances in the soil solution such protection should be credited can not be stated definitely. The slime, mentioned by Van Suchtelen (10, p. 154), may be of influence in this connection.

TABLE II.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Broth.	Milk.	Soil solution.
March 18..	1, 100, 000	1, 500, 000	1, 440, 000	1, 613, 000	1, 024, 000	1, 176, 800	1, 460, 000
26..	−10, 000	−10, 000	10, 125, 000	−10, 000	19, 967, 000	185, 000	40, 000
April 6....	−25	25	50	−25	220, 000	405, 000	8, 600
17....	−25	−25	−25	−25	−25	−25	−25

An additional experiment was conducted employing the same cultures used in the previous experiments. The procedure was the same, except that as a basis for quantitative determinations two samples were taken from each set instead of one. As the plates from several of the flasks showed no colonies whatever on May 3, even in the lowest dilutions, which represented 1/25 gm., it was thought advisable in making the next determinations, on May 13, to take 1-gm. samples from these flasks and mix them directly with the melted medium in the Petri dish instead of plating 1 c. c. of a dilute suspension as previously done. It is quite evident that the direct mixture of the sand with the plating medium tends to give higher counts than those secured by plating the washings of the sand, for in the latter case a large number of organisms undoubtedly remain attached to the sand particles instead of being washed off into the suspension. This difference in technic may account for the apparent increase in numbers in certain cases, as shown by the last plating.

TABLE III.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Albumin solution.	Broth.	Milk.	Soil solution.
April 16	1,648,000	2,144,000	1,901,000	3,234,000	360,000	1,477,000	4,026,000	1,266,000
May 3	—25	25	—25	—25	56	428,625	515	391
13	.....	.....	116	.....	2	432,000	106	3,080

The figures in Table III offer little except a general confirmation of the results of the two other experiments. As the sand was air dry after April 26, it may be understood that the counts on May 3 and May 13 represent the numbers surviving 7 and 17 days desiccation, respectively. Attention must be called to the fact that the lack of figures to show the comparison in increase of bacteria in the different solutions between April 16 and April 26 makes it impossible to overlook entirely the function of these different solutions in their nutritive capacity. Plates were made on April 26, but the nitrogen-free agar made up with maltose instead of saccharose proved an unfortunate choice; for no colonies whatever developed, although, as seen by the two subsequent platings, living organisms were then present in abundance. However, the favorable influence of the soil solution, whether it may be as a food material for soil organisms or a protection during desiccation, can not be disputed.

An experiment was conducted to compare the longevity of *P. radicicola* dried in quartz sand and in clay-loam garden soil. As in the foregoing experiments, the organism was grown for five days at room temperature on nitrogen-free ash agar. The bacterial growth from one agar slant was transferred to 12 c. c. of physiological salt solution and the mixture shaken thoroughly, and 1 c. c. of the suspension was diluted and plated quantitatively. To the two flasks each of clay loam and quartz sand were added 2 c. c. of the bacterial suspension. The clay loam had been sifted and air dried. The quartz sand had been prepared after Rahn's method, described previously. Fifty-gm. portions of each were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and sterilized by heating in the autoclave for 45 minutes under 15 pounds' pressure.

The inoculated flasks were shaken to distribute the organisms throughout the sand or soil, and then kept in a dark, well-ventilated place at a temperature of 22° to 25° C. The number of living organisms per gram of sand and loam was determined at intervals by plating quantitatively from two samples of each.

TABLE IV.—*Difference in longevity of Pseudomonas radicicola dried in quartz sand and in clay-loam soil*

Date.	Sand.	Clay loam.
April 16	1,648,000	1,648,000
May 3	25	42,133
13	.....	33,025

It is evident from the data above tabulated that a larger number of organisms survive a limited period of desiccation in clay loam than in quartz sand. This may be partly explained by the difference in grain size and hygroscopic moisture of the two. A given weight of coarse quartz sand consisting of large particles has a surface much less than that of the same quantity of finely divided garden soil, and it therefore retains a much smaller amount of moisture in the hygroscopic form. If the grain size were the only distinction between sand and clay-loam soil, it might properly be concluded that the longevity of organisms in such materials is directly proportional to the percentage of hygroscopic water retained. Such a conclusion is not permissible, however, for the clay-loam soil differs from the sand not only in texture but in content of organic constituents. The amount of such material in any sand is small, and in this case, where the sand was treated with acid, it may be regarded as having been absent. The experiments already described indicate that the soil solution contains substances which offer to the bacteria some protection against desiccation. The soil solution used in our experiments was extracted from just such a soil as was used in the experiment now under discussion.

A further experiment was conducted to compare the changes in numbers and kinds of organisms when soil solution is dried in different types of soils. Soil solution extracted from a rich garden loam was used for this experiment. The soils, obtained from the Soil Physics Department of the Michigan Agricultural College, were of five different types: Muck, sand, sandy loam, clay, and clay loam.

Fifty-gm. portions of these soils in the air-dry condition were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and were then sterilized in the autoclave for 45 minutes under 15 pounds pressure. For greater exactness the total quantity of soil solution was agitated and then divided into five 250 c. c. portions; from each of these 1 c. c. was plated on ordinary agar in dilutions of 1 to 10,000, 1 to 100,000 and 1 to 1,000,000. Ten flasks of each type of soil were then inoculated with the soil solution, all the solution used for any one type of soil being taken from a single flask. Although it was desired to have the inoculum approximately equal in all cases, a quantity of liquid which barely moistened the muck and clay loam was found to more than saturate the coarser soils. So, to make the physical conditions more nearly alike, 15 c. c. of the solution was used for each flask of clay, clay loam, and muck, but only 10 c. c. for the flasks of sand and sandy loam.

The inoculated flasks were kept on a shelf in the laboratory at a temperature of 20° to 25° C., exposed to very dim diffused light, and subject to the influence of normal variations in the humidity of the room atmosphere. At intervals of about four weeks quantitative determinations were made, samples being taken from two flasks of each soil. After the first plating, samples were taken from one flask opened at the previous

plating and from one new flask each time, the object being to secure more representative counts. Plates were made with ordinary agar and kept for one week at a temperature of 22° to 25° C. before counting.

Moisture determinations were made in duplicate at the time of each quantitative plating, the bacterial counts being then computed on the oven-dry basis.[1] Small variations in the percentage of moisture, occurring after the soils attained the air-dry condition (which with sand and sandy loam was by March 3 and with the other three soils between March 3 and March 29), are probably the result of fluctuations in the humidity of the room air. In the case of clay it was impossible to secure a thoroughly mixed sample, owing to its drying into a sort of hard, baked condition; therefore, a slight irregularity in the moisture determinations could not be avoided. The data are recorded in Table V.

TABLE V.—*Number of bacteria per gram in 50 grams of sand, sandy loam, clay, clay loam, and muck when dried after the addition of soil solution*

Date.	10 c. c. of soil solution added.				15 c. c. of soil solution added.					
	Sand.		Sandy loam.		Clay.		Clay loam.		Muck.	
	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Per centage of water.	Number of bacteria per gram.	Percentage of water.
1914:										
Nov. 17	285,200	20.0	170,000	20.0	462,900	30.0	225,000	30.0	453,900	30.0
Dec. 29	4,318,000	14.54	26,170,000	14.38	11,500,000	28.96	60,840,000	31.81	33,689,000	26.13
1915:										
Jan. 28	1,912,000	6.25	5,806,000	2.81	1,492,000	19.17	26,006,000	16.96	16,613,000	24.85
Mar. 3	197,000	.1	1,555,000	.84	914,000	3.59	12,798,000	9.83	5,782,000	19.51
29	51,900	.36	1,967,000	.78	552,000	.93	4,659,000	2.93	4,924,000	16.33
Apr. 21	18,900	.16	1,066,000	.84	447,100	1.57	4,135,000	3.31	4,217,000	16.32
May 7	32,500	.27	983,000	1.08	278,800	1.81	3,845,000	3.65	2,220,000	16.25
14	37,000	.22	2,245,000	1.10	378,000	1.74	3,914,000	3.63	2,703,000	15.91
June 18	41,000	.20	3,218,000	1.22	494,000	1.98	5,456,000	4.26	1,836,000	16.80
Sept 6[a]	127,600	.14	6,523,000	1.23	1,241,000	2.26	11,686,000	4.30	2,781,000	16.78

[a] This count was made by Mr. O. M. Gruzit, Graduate Assistant in Bacteriology.

With a view to determining the predominant types of organisms placed in the soils, isolations were made from a few of the most common types of colonies occurring on the plates of the original soil solution. The characteristics of these organisms were studied. It must not be assumed, however, from the fact that so few organisms were isolated, that the flora of the soil solution was limited to the species observed. The high dilutions necessary for obtaining accurate quantitative plates failed, of course, to show up the organisms which were present in smaller numbers. From the quantitative plates made after the soils reached the air-dry state, between March 3 and May 7, isolations were made of the most numerous types. As the muck plates were frequently overgrown with a downy white mold, but few pure cultures could be obtained from

[1] Dried at 105° C. for 24 hours.

that source. As seen in Table V, the loam soils and muck show a higher count six months from the time of inoculation than do the clay and sand. During the first six weeks all five soils contained an amount of moisture sufficient for bacterial growth, and during the last two months only were the soils in the air-dry state. The amount of activity in the period intermediate between the optimum and minimum supply of moisture shows a gradual decrease, the rate varying in the different soils.

While there was not a great difference in the initial counts, the opportunity for bacterial growth in the five types of soil was by no means the same. This is clearly evidenced by the contrast between their counts during the first period, when the moisture content was yet sufficient to permit multiplication. Since the sand was saturated with the amount of soil solution used as an inoculum, it at first presented conditions more favorable to anaerobic than to aerobic species. As this amount of moisture diminished and the oxygen supply increased, opportunity for the growth of aerobic types was given, but the extent of this favorable period was limited not only by the small amount of organic food material but also by the extremely rapid evaporation of moisture. Conditions in the clay were at first comparable with those in the sand, it being practically waterlogged. With the gradual reduction in moisture and increase in aeration, the growth of aerobic and facultative bacteria proceeded. The smaller size of the grains produced two noticeable effects—viz, a limited oxygen supply, inhibitory to the extensive multiplication of aerobic species, and a prolonged retention of moisture, which favored the longevity, if not the activity, of non-spore-bearing bacteria. As in the sand, a low content of organic nutrients acted as a natural limit to the growth of saprophytic species. In the clay loam, sandy loam, and muck multiplication was possible from the start, for the amount of solution used for inoculation was just sufficient to moisten the soils without saturating them. Their higher content of organic substance also gave them an advantage in respect to nutrition.

However, in these soils also differences in size of grain, thickness of moisture film, and oxygen supply proved to be factors of more influence than the mere abundance of organic food substance. The muck, for instance, although containing the highest percentage of such organic materials, proved to be of a less favorable medium for bacterial growth than did the clay loam. The grain size of the clay loam appeared to be that which was most advantageous with respect to aeration, thickness of moisture film, and retention of hygroscopic water. Its content of decomposable substances, while not so great as that of the muck, was more than sufficient for microbial development. The sandy loam, with a smaller amount of organic materials, somewhat larger grain size, and consequently less hygroscopicity, did not show as large numbers of living

organisms at any time as did the clay loam, although its oxygen supply in consequence of these same conditions must have been somewhat greater.

We therefore perceive that the optimum condition for microbial activity in soil is a proper adjustment of these previously mentioned factors. With regard to longevity, fewer factors are concerned, the data so far obtained indicating that it is a function of both grain size (and therefore amount of hygroscopic moisture) and content of organic substances.

The influence of soil type was made evident not only in the numerical counts but also in the varieties of organisms persisting in the different soils throughout the two months during which they were in the air-dry state. As the condition of the sand had been such as to favor the development of organisms with high oxygen requirements, plates of high dilution always showed a predominance of those types. Such of these as were spore bearers became a larger and larger proportion of the total number, as the period of desiccation extended and the non-spore-bearing species died out. Among the spore bearers most frequently found were *Bacillus mycoides* and aerobes of similar morphological and cultural characters. Of the non-spore-formers an organism found in larger numbers than any other single species showed the greatest longevity. The characteristics of this organism are as follows:

It is a rod with rounded ends, 0.6μ by 1.3 to 1.5μ. It is actively motile, non-spore-forming and non-capsule-forming. It is frequently observed in pairs. It stains readily with aqueous alcoholic fuchsin. In nutrient broth it produces a decided turbidity, some sediment, and a soft surface scum. The growth on agar is glistening, translucent, grayish white, and very abundant. On a gelatin stab there is a white surface growth, with a filiform growth in the stab, but not liquefaction. Litmus milk becomes bluer after 48 hours; some peptonization in 30 days. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Facultative anaerobe. Optimum temperature, 25° C. Habitat, soil.

Physical conditions in the clay had somewhat inhibited the extensive multiplication of strongly aerobic types, but permitted the development of facultative bacteria. Since anaerobic organisms could not be secured by the method of plating used, no mention of them is possible. As the non-spore-bearing types declined, the plates showed more evidence of spore-bearing, strictly aerobic varieties similar to those met with in the sand. The fact that such colonies were not found until their diminishing numbers necessitated the use of lower dilutions suggests their development from spores which had merely remained latent in the clay without passing through a process of multiplication and subsequent destruction like the majority of the facultative non-spore-bearing species. The non-spore-forming organism showing greatest endurance of desiccation was a type identical with that persisting in the sand.

During the period of extensive multiplication, the plates from sandy loam, clay loam, and muck showed quite similar types, although the sandy loam has slightly greater numbers of the strongly aerobic spore-forming species. As the numbers diminished, spore-bearing types became more frequent on plates from both sandy loam and clay loam, but were not evident on the plates from muck. It is to be inferred that the multiplication of those in the finest soil had not progressed to such an extent as to make their colonies numerous in high dilutions, their numbers apparently being in proportion to the grain size and amount of aeration. The most persistent non-spore-bearing organism was of the type already referred to, as found in clay and sand. In addition to this, certain chromogenic cocci and one variety of slime-forming organism were frequent on plates from all three of these soils through the time of desiccation. This slime former, which was especially numerous on plates from muck, is described as follows:

The organism is a rod 0.4μ by 0.6 to 0.7μ; nonmotile. No spores observed. No capsule demonstrated. Stains readily with aqueous alcoholic fuchsin. Nutrient broth made slimy and very turbid. Growth on agar spreading, translucent, orange-yellow, slimy. Gelatin stab, surface growth and rapid liquefaction. Litmus milk discolored, alkaline, slimy; peptonization begun in 48 hours and complete in 10 days. Facultative anaerobe. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Habitat, soil.

Attention should be called to the rather peculiar circumstance that not one of the organisms isolated during the last two months corresponds to any one of the four organisms which predominated in the original soil solution used for the inoculation of the five soils. The extinction of these species may have been due either to the unfavorable influence of association with other organisms during the period of active multiplication or to their lack of endurance when supplied with less than the optimum amount of moisture.

CONCLUSIONS

(1) The survival of non-spore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain size and hygroscopic moisture.

(2) Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand, under the conditions of our experiment.

(3) If bacteria are suspended in the solution extracted from a rich clay loam before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in physiological salt solution.

(4) The solution extracted from a rich clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

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OBSERVATIONS ON THE LIFE HISTORY OF THE CHERRY LEAF BEETLE

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INTRODUCTION

The cherry leaf beetle (*Galerucella cavicollis* Lec.), which attracted much attention during the season of 1915, is a native insect that has adopted several new food plants, at least in the beetle stage. Not since the first record of its work on cultivated plants, in 1894, has its injury been as great or as widespread as during the summer just past. It would seem that the early prediction of Davis (2),[1] who first recorded the beetle's work on cherry (*Prunus* spp.), was about to be fulfilled, that as it was a northern and widespread species we might expect it to become increasingly injurious from year to year.

HISTORICAL REVIEW

The cherry leaf beetle was originally described by Le Conte in 1865 (5, p. 216) from a single specimen received from North Carolina. Nothing further is recorded of this beetle till 1890, when Packard, who found this species in large numbers at Berlin Falls, N. H., eating holes in the leaves of wild cherry, probably the pin cherry (*Prunus pennsylvanica*), refers (7, p. 529) to it under the name "*Galeruca sanguinea.*"

The next reference is by Davis (2), who reports it as being abundant at Bellaire, Mich., during the summer of 1894 and destroying the foliage of cultivated cherries. This is the first record of this beetle's attacking the foliage of cultivated trees, and Davis makes the suggestion that as this insect is a northern species it may yet become quite injurious. The larvæ were found in this same locality; but it is not stated on what plants they were feeding, though the writer states that wild cherries were only a short distance away.

Lintner (6) records this beetle as occurring in thousands on June 10, 1895, at Ausable Forks, N. Y., feeding on the foliage of the cherry left uninjured by late frosts. He also states that his correspondent found this same insect at work early in July on the foliage of young chestnut trees, but that he did not verify this observation.

Felt (3), in 1898, records outbreaks of this insect at Corning, N. Y., the beetles occurring in such numbers as to threaten the destruction of the trees. Smith was the first to record the occurrence of this beetle on peach, having found it in Pennsylvania during the summer of 1898.

[1] Reference is made by number to "Literature cited," p. 949.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
ch

Vol. V, No. 20
Feb. 14, 1916
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Johnson (4) reports an extensive outbreak on "fire cherry" (*Prunus pennsylvanica*) at Ricketts, Wyoming County, Pa., during September, 1897, the beetles and larvæ occurring in immense numbers.

Chittenden (1) reports outbreaks of this beetle in June, 1898, at St. Ignace, Mich., on cherry and at Spruce Creek, Huntington County, Mich., on young peach trees. He states that larvæ are known to feed on cherry and probably also on peach, but mentions no definite records of such occurrences on the peach.

Since the publication of Chittenden's article, nothing has been recorded of this insect, and undoubtedly during all the years since 1898 no injury of any consequence has been committed by it.

OUTBREAKS IN NEW YORK IN 1915

During the summer of 1915 several severe outbreaks occurred in New York, the beetles defoliating cherry, peach (*Amygdalus persica*), and plum (*Prunus* spp.). On June 3 Mr. E. P. Putnam, of Jamestown, N. Y., wrote the Entomological Department, inclosing specimens of beetles, saying that they were defoliating wild cherry and peach trees in the park and also reported them as seriously defoliating cherry and peach trees throughout the town and neighboring districts. On June 11, Mr. H. B. Rogers reported them as injuring cherry and peach and later wrote that this beetle had done considerable injury throughout Chautauqua County. Reports of injury have been received from the following localities: Sonyea (cherry, peach, and plum); Perry, Scio (cherry); Olean, Honeoye Falls (cherry); Bath (cherry); Holland (cherry); Collins, Gowanda, Wyoming, Batavia (cherry and peach); Perrysburg (cherry); Jamestown (cherry and peach); Chautauqua (cherry and peach); Kennedy, Fredonia, Ripley (plum and peach); Castile (cherry); Elmira (cherry and peach); Hornell (cherry); and Ithaca (cherry and peach). All these reports came during the month of June and early in July and nothing has been heard of later injury. Evidently the beetles have not bred locally in such numbers that the work of the adults would attract attention in August and September.

The causes which brought about so widespread an outbreak of this insect can not at present be determined. Practically all the injury was restricted to western and southwestern New York. It has been suggested that the beetles migrated northward from Pennsylvania, but this does not seem plausible, as the native host, *Prunus pennsylvanica*, is a northern tree, occurring southward only as far as Pennsylvania and in the mountains to North Carolina and Tennessee. Conditions must have been favorable for the increase of this beetle in 1914 and hibernation must have been attended with little loss of life, resulting in such large numbers of the overwintering beetles as to cause overcrowding of the normal food plants. Should favorable conditions prevail during any year, we may again look for a sudden and perhaps more widespread outbreak.

LIFE HISTORY AND HABITS

The cherry leaf beetle is a pretty, dull-red beetle measuring 4.5 to 5.5 mm. in length (Pl. LXIV, fig. 1). The antennæ are black, and the legs vary from almost black to nearly reddish in color. There are no strikingly distinguishing characters, but the coloring will nearly always serve to separate it from the more closely related northern species. The beetle is widely distributed, occurring from Canada through the New England States southward into Pennsylvania and west to Wisconsin. Chittenden (1) also records it from Texas and Vancouver, British Columbia. The original specimen described by Le Conte (5, p. 216) is from North Carolina.

This insect is one of our native beetles and up to 1894 had only been recorded on wild cherry. In that year it was found attacking the cultivated cherry, destroying the foliage. Later Smith (8) recorded it as injuring peach, and this year it has been reported as feeding on plum. How much more extended the feeding habits of this beetle may become can not even be guessed, though its future destructiveness will depend largely upon whether the larvæ can also adapt themselves to new and closely related food plants.

The beetles pass the winter in hibernation and, although the time of emergence has not been determined, they probably appear in May or, if the weather is favorable, during the latter part of April. They feed actively during May and June not only on the pin cherry but also on the peach, cherry, and in some instances the plum (Pl. LXV, fig. 5). In the field the beetles began to leave the cultivated food plants early in July and practically all had gone by the middle of the month.

In New York State there is only a single brood a season. The new brood of adults appears during the second week in August and becomes common during the latter part of the month and early September; they feed almost exclusively on the pin cherry and do not seem to migrate far from their host plant. In our rearing cages they began entering the soil or crawling under stones about the middle of September, but on fine days would return to feed on the pin-cherry foliage. In early October they had all entered hibernating quarters and did not leave them even on the finest or warmest days.

The work of the beetles is most noticeable during June and early July. After the middle of July the beetles had largely disappeared from the cultivated trees about Ithaca. Although many adults had been seen in copula, no eggs were observed, despite a close watch on all their new food plants. It was supposed that in accordance with the habits of closely allied species, as the elm leaf beetle (*Galer cella luteola*), the eggs would be found on the host plant.

On July 21 Mr. Cotton, a student in the Entomological Department, found adults and what he considered larvæ of this species on pin cherry.

On examination it was at once seen that there were larvæ in all stages, but the closest search did not reveal a single egg on the foliage or trunk or branches of the tree. The youngest larvæ, which seemed to us to have just hatched, were very active, running about over the trunk and branches, and a search at the base of the trees soon revealed immense numbers of eggs just below the surface of the soil, in the matted grass, under sticks, and among rubbish.

THE EGG

We did not observe the date of the first egg laying nor determine the number of eggs laid by a single female. At Ithaca egg laying occurs from June to August. If we judge from the length of the larval life and the egg stage, the deposition of eggs at Ithaca undoubtedly began the last week in June. The egg-laying period extended throughout July and the early part of August.

The egg is entirely different in shape from that of closely allied species. It is oval, of a light-straw color, and measures 0.72 to 0.84 mm. in length by 0.60 to 0.64 mm. in width. The entire surface is marked with rather regular hexagonal areas. Large numbers of these eggs were found at the base of the few pin-cherry trees located close to the Cornell University grounds. The eggs adhered rather firmly to each other and to the matted grass. Although close search was made, no eggs could be found at the base of any other species of Prunus (Pl. LXV, fig. 1, 2).

THE LARVA

During the latter part of July eggs hatched in from 14 to 18 days after they were laid. The young larva escapes from the egg by cutting a hole through one side with the mandibles. The young larvæ are very active, running about rapidly. They soon find their way to the trunk of the tree and could be seen any time during the hatching period clambering actively over the branches in search of the young and tender foliage near the tips of the twigs. They are found most commonly on the under surface of the foliage skeletonizing the leaves. They feed ravenously, grow rapidly, and reach maturity in from two to three weeks. Where the larvæ are abundant all the foliage may be so completely skeletonized as to turn brown and die, giving the trees a scorched appearance (Pl. LXV, fig. 3, 4). The length of the life cycle, with the number of molts, is given in Table I.

TABLE I.—*Length of life cycle and number of molts of the cherry leaf beetle*

Eggs.		First stage.	Second stage.	Third stage.	Entered soil to pupate.	Emergence of adult.
Laid.[1]	Hatched.					
.....	July 23	July 30	Aug. 3	Aug. 9	Aug. 10	Aug. 28
.....	...do.....	July 29	...do.....	...do.....	...do.....	Do.
.....	...do.....	July 30	Aug. 4	Aug. 8	Aug. 9	Aug. 26
.....	...do.....	July 28	Aug. 1	Aug. 4	Aug. 5	Aug. 24
.....	...do.....	July 27	...do.....	Aug. 6	Aug. 7	Aug. 25
.....	...do.....	July 29	Aug. 3	Aug. 8	Aug. 9	Aug. 27
.....	...do.....	...do.....	Aug. 1	Aug. 7	Aug. 8	Aug. 26
.....	...do.....	July 30	Aug. 2	...do.....	...do.....	Aug. 27
.....	...do.....	July 28	...do.....	...do.....	...do.....	Aug. 28

[1] From another series of experiments the length of the egg stage was determined. The eggs hatched as follows: 15, 18, 17, 18, 16, 18, and 14 days after they were laid. The average is 16 days. If this were taken as the average length of the egg stage, the total length of the life cycle from the egg to the adult would vary from 48 to 53 days.

DESCRIPTION OF LARVAL STAGE

FIRST INSTAR.—The newly-hatched larva is depressed, fuscous in color, the head, thoracic shield, legs, and anal segment, black. Scattered over the larva are a number of setæ. Length, 1.4 to 1.6 mm.; greatest width, 0.45 to 0.50 mm.

SECOND INSTAR.—Nearly cylindrical, slightly depressed, fuscous to brown in color, the head, legs, thoracic and anal shields black. The ground color is almost entirely obscured by the black areas as shown in Plate LXIV, figure 2. On each segment, except the prothoracic and anal, there are two oval, rather sharply defined, large, black areas separated from each other by a narrow line. Laterad of the black areas are angular black markings as shown in Plate LXIV, figure 2. Length, 2.5 to 3.5 mm.

MATURE LARVA, THIRD INSTAR.—Length, 6 to 8 mm., nearly cylindrical, somewhat depressed, with an average width of about 2 mm. (Pl. LXIV, fig. 3). The larva after the second molt measures 5 mm. in length and is black in color. As it feeds, the black spots and markings become separated and the brownish yellow ground color shows distinctly. Head black, narrower than thorax; mouth parts yellowish brown. Legs, prothoracic and anal shields black. Dorsally each segment, except the prothorax and anal segments, with two sharply defined oval to rectangular black areas separated by a brownish yellow line; laterad of each of these there is an angular black spot and beyond each of these a smaller rounded black mark. Along the lateral margin there is an elongate oval black spot on each segment. The venter of each abdominal segment is marked with five dark brown to black spots, the central one being largest. The prosternum is black; meso- and meta-sterna each with a narrow, elongate, black area in front and two black rounded spots just caudad of it.

FOOD HABITS OF THE LARVA

From a close examination about Ithaca we failed to find the larvæ present on any trees but the pin cherry. The few trees of this species located near the campus were swarming with the beetles and larvæ. However, on the other food plants of the adult we found, late in the season, only a few beetles and no larvæ. To determine whether the larvæ could survive and reach maturity on the other species of Prunus the following experiments were performed:

EXPERIMENT 1.—On July 23 six larvæ, some almost mature, were placed on the leaves of *Prunus avium*. Two died on July 25, two more on the 27th, and the remaining two entered the soil to pupate on July 28, the adults emerging on August 15. The immature larvæ did not feed, but the nearly mature forms fed slightly before entering the soil to pupate.

EXPERIMENT 2.—On July 23 two young larvæ were placed on leaves of *Prunus avium*. Both died on the 26th without having fed at all.

EXPERIMENT 3.—On July 27 three half-grown larvæ were placed on leaves of *Prunus virginiana*. On the 28th all had left and entered the soil in an attempt to pupate. Later all failed to pupate and died.

EXPERIMENT 4.—On July 27 five half-grown larvæ were placed on leaves of *Prunus virginiana*. On July 28 one was dead and the others entered the soil. All failed to reach maturity.

EXPERIMENT 5.—On July 28 three half-grown larvæ were placed on leaves of *Prunus serotina*. All failed to feed and died on July 31. On the same day four more half-grown larvæ were placed on leaves of *P. serotina*. All failed to feed and died on July 30.

It will be seen from the above experiments that the larvæ seem to be unable to survive on either the cultivated sweet cherry (*Prunus avium*) or the common two native varieties *P. serotina* and *P. virginiana*. It is unfortunate that through an oversight experiments were not made with the other species of Prunus. The food plants of the larvæ are undoubtedly restricted at the present time to the wild red, or pin, cherry. Whether the larva can succeed in adapting itself to other host plants seems to be a doubtful question, so that in the future the abundance of the beetles will depend not so much on the presence of its enemies as on a goodly supply of the larval food plant.

THE PUPA

Pupation takes place at or slightly below the surface of the soil. No special preparation is made by the larva, the pupa often lying openly on the surface in the grass or under rubbish. The pupa is bright yellow, strongly convex, without any distinguishing markings. Scattered over it are small, short brownish tipped setæ, which aid in preventing injury from the soil. The tip of the abdomen is furnished with two diverging strong black spines (Pl. LXIV, fig. 4).

CONTROL OF CHERRY LEAF BEETLE

On account of the comparatively small numbers of the beetles at Ithaca, we were not able to conduct control experiments. However, several of our correspondents have had good success with lead arsenate (paste) used at the rate of 4 to 5 pounds to 100 gallons of water and also with a spray containing 40 per cent nicotine. In the case of the nicotine spray our correspondent used it at the rate of 3 pints to 100 gallons of water and reported good success. He also reports failure with lead arsenate, though using treble and even quadruple the quantities generally recommended for foliage-feeding insects.

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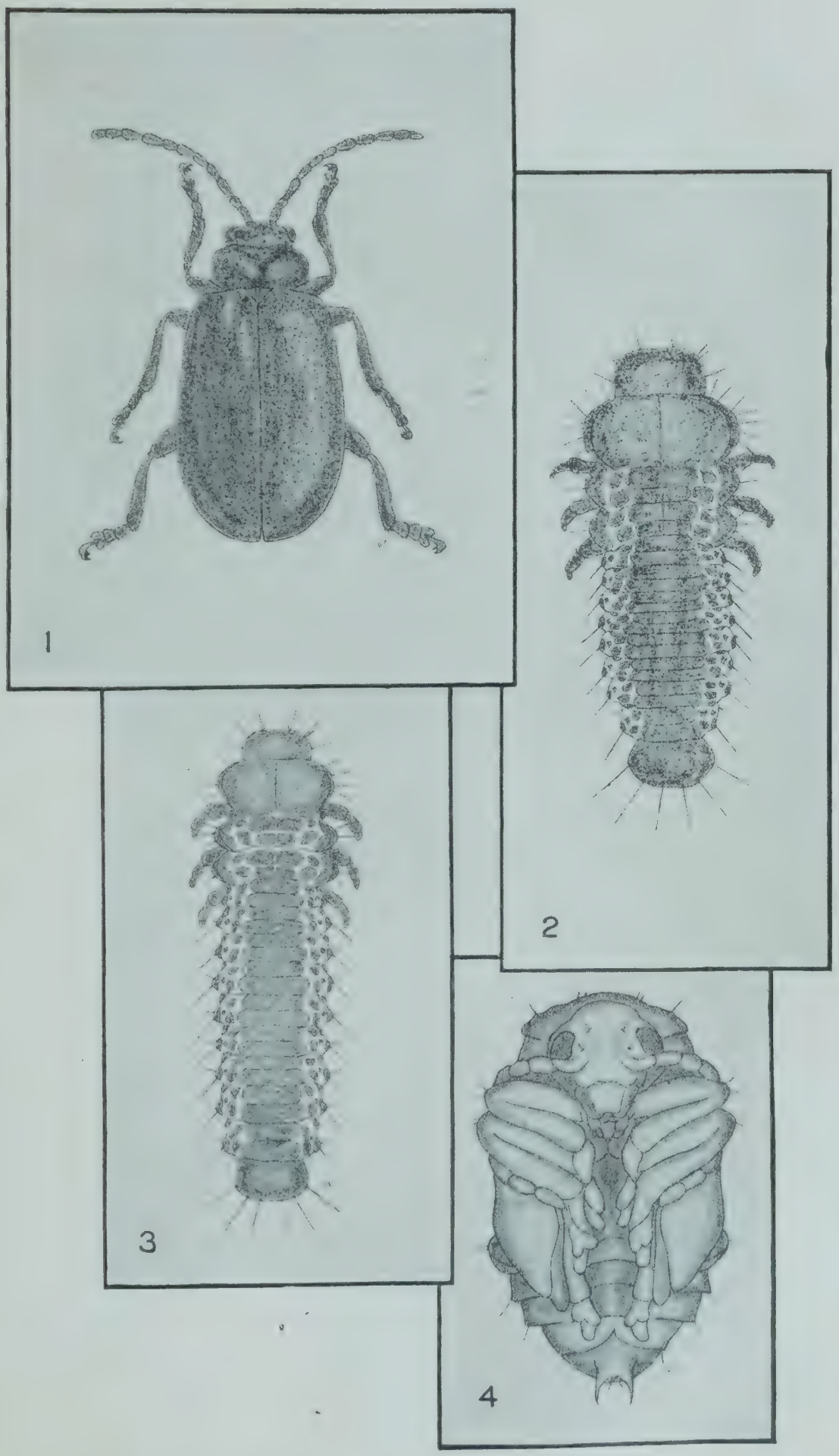
1890. Insects injurious to forest and shade trees. U. S. Dept. Agr. Ent. Com. 5th Rpt., 928 p., 306 fig., 40 pl. (partly col.).

(8) SMITH, J. B.
1898. [Differences in the habits of insects in neighboring states.] *In* U. S. Dept. Agr. Div. Ent. Bul. 17, n. s., p. 23.

PLATE LXIV

Galerucella cavicollis:

Fig. 1.—Adult.
Fig. 2.—Larva, second instar.
Fig. 3.—Larva, third instar.
Fig. 4.—Pupa.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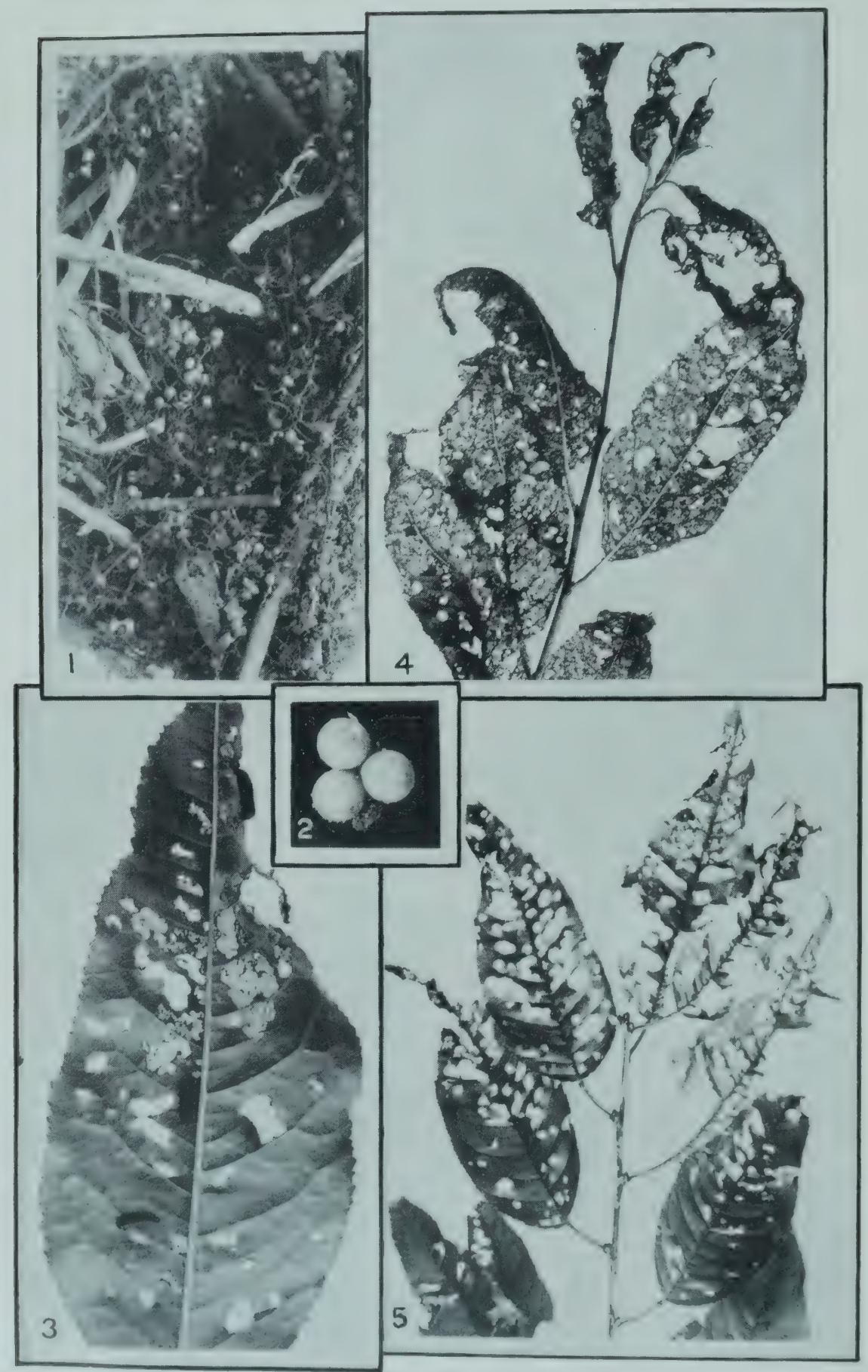
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APPARATUS FOR MEASURING THE WEAR OF CONCRETE ROADS

By A. T. Goldbeck,

Engineer of Tests, Office of Public Roads and Rural Engineering

Many miles of concrete roads have been built during the past few years, and the methods employed in their construction are rapidly becoming standardized. The concrete mixture is now made comparatively rich, and in general the aggregates are selected with as much care as present knowledge of these materials permits. Even yet, however, it is doubtful whether the right mixture is being used for the purpose: Whether it is too rich for economy or whether it should be made still richer. It is questionable what kinds of coarse aggregates give the most economical results: Whether they should be composed of hard, tough fragments of trap rock or of softer, more friable pieces of limestone of approximately the same degree of hardness as the mortar in which they are embedded; whether angular fragments of crushed stone should be used or whether round pieces of gravel are equally satisfactory. Definite knowledge on these points based on scientific information seems to be lacking.

The ideal concrete road should wear uniformly and slowly. When due care is exercised in construction and the necessary precautions are taken in maintenance, uniformity of wear may to a large extent be controlled. But little is known about the rate of wear of concrete roads having various aggregates and carrying different kinds of traffic. General observation indicates that some roads with particular kinds of aggregates are wearing more slowly than others containing different coarse aggregates, even though the traffic conditions are nearly alike. We have, however, no definite idea of the amount of wear in these different roads. There must come a time in the life of every concrete road when, notwithstanding careful maintenance through crack protection and patching, its thickness will approach the minimum, making imperative the expenditure of a considerable amount of money for a new wearing surface to replace that gradually worn away by traffic. Every fractional part of an inch decrease in thickness therefore represents a very definite depreciation in the value of the pavement. Money can not be expended intelligently on various aggregates mixed with cement in different proportions for road construction without accurate knowledge of one of the most important factors governing the expenditure—namely, the probable rate of depreciation of the road as determined by actual wear.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 20
Feb. 14, 1916
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This consideration has led the Office of Public Roads and Rural Engineering to attempt to gain definite knowledge of the wear of concrete roads carrying various kinds of traffic, and a special instrument has been designed by the writer and built in this office for that purpose. Several methods of taking autographic records of the cross section of the road were considered, but were discarded in favor of the simpler and more portable form of instrument finally constructed.

Essentially, this instrument consists of a fine wire stretched tightly across the road at a constant height, together with an inside micrometer for measuring the distance from the road surface to the wire. Measurements taken 1 foot apart across the road permit the plotting of its cross section, and if these measurements are repeated at long intervals the change of cross section or the decrease in the thickness of the road will be revealed.

The accompanying illustrations show the instrument in detail and its method of application on the road. If Plate LXVI, figure 1, and text figure 1 are first referred to, the component parts of the apparatus may be seen very plainly.

Pieces *A* and *B* are made of cement mortar and have embedded in them steel rods, *C*, drilled with holes slightly inclined with the horizontal. A fine piano wire about 0.01 of an inch in diameter is passed through these holes and is stretched across the road from block *A* to block *B*. The tops of these rods are each provided with a disk-level bubble, so that when placed in position in the road the rods may be adjusted to a vertical position. Block *A*, which is heavier than block *B*, is provided with two adjusting screws, *D*, for adjusting rod *C* to the vertical. Block *B* rests on two points only, one the lower end of rod *C* and the other the end of adjusting screw *D*. Constant tension is produced in the wire by the weight of block *B*, which is pivoted about the bottom of rod *C* and is adjusted to a horizontal position by means of rack *E*, provided at the end of the wire. As the weight of block *B* is constant, the tension in the wire, and consequently the amount of sag for like spans, must remain the same. A very definite and fixed datum is thus provided, which should remain constant from year to year and which is very easily established by merely placing the end blocks of the apparatus in their proper position on the road.

The bottoms of rods *C* are spherical in shape; and when in use on concrete roads, they rest on the flat tops of bronze plugs cemented in the road surface. These plugs are ½ inch in diameter and are 1¾ inches long. They are set ¾ inch below the surface, and their tops are protected by means of a brass pipe plugged with a bituminous-sand mixture during the long intervals between readings.

In obtaining the wear measurements a chalk line is first snapped across the road between the bronze plugs, and the points at which it is

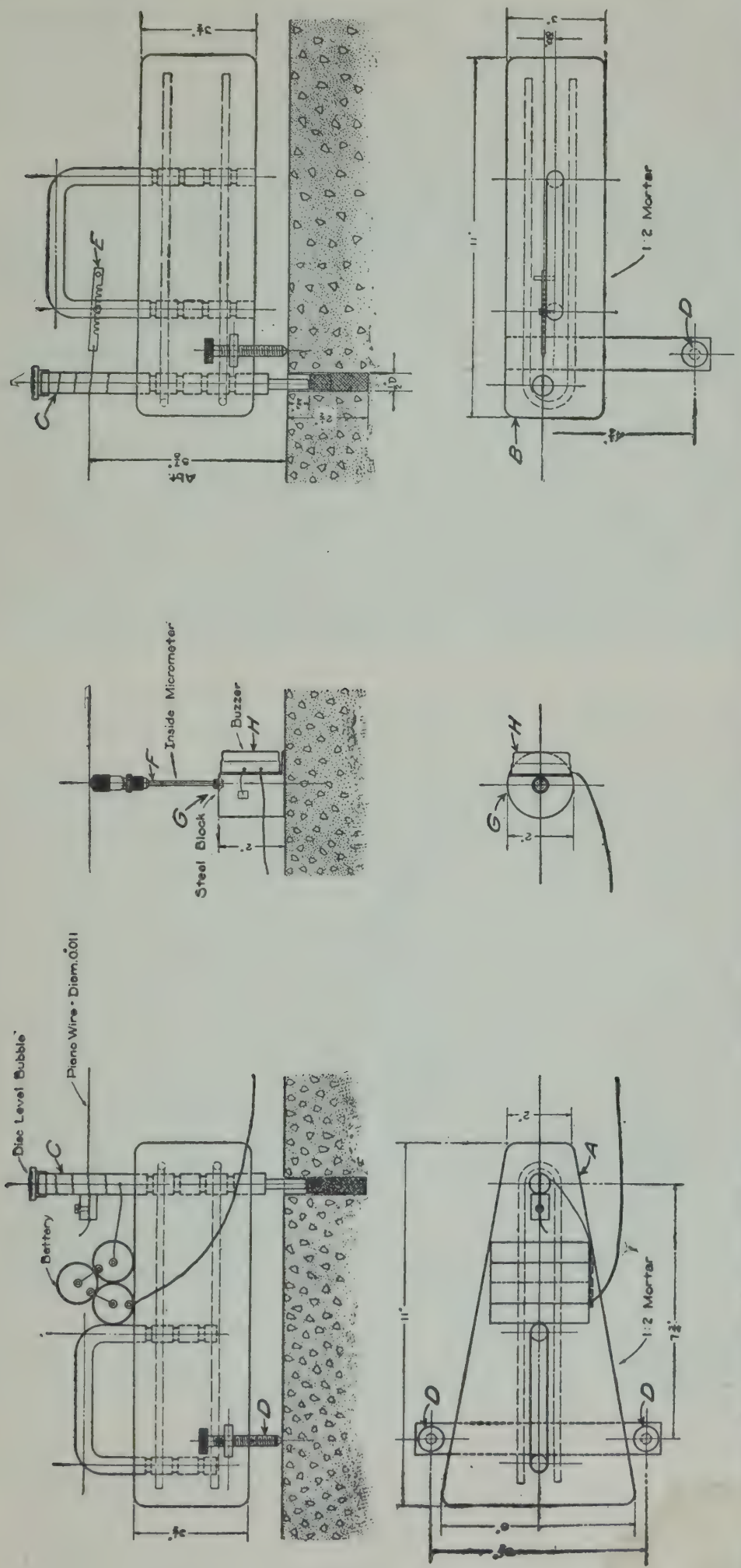

FIG. 1.—Details of instrument for measuring the wear of roads: *A*, Heavy mortar block; *B*, light mortar block; *C*, steel rod; *D*, level adjusting screws; *E*, adjusting rack; *F*, inside micrometer; *G*, steel bearing block; *H*, electric buzzer.

purposed to take readings are marked on this line. At these points a steel block, *G*, 2 inches in diameter, is placed, in order to avoid measuring the small local inequalities in the road surface. In the top of this block a flat-bottomed cylindrical recess is made, and an ordinary inside micrometer is held in the recess, while its upper end is adjusted to contact with the steel wire stretched across the road. An electric buzzer, *H*, is mounted on the side of this block, and when contact is made between the micrometer and the wire an electric circuit is completed through the buzzer. With this instrument readings for wear may be taken to the nearest 0.001 inch, although this degree of accuracy will not be necessary.

Holes in the road in which the bronze plugs are set are drilled by means of a special hand-operated drill press carrying a star drill.

In Plate LXVI, figure 2, the method of mounting the apparatus in the road and its manipulation are plainly shown. On the left is the heavier end block carrying the batteries, and on the right is the lighter block the weight of which supplies constant tension to the fine steel wire, part of which is seen in front of the operator. The cord extending on the road surface from the heavier block to the small steel block carrying the micrometer is one of the leads from the battery to the electric buzzer.

Placing the buzzer in this position near the operator obviously is advantageous, especially when the instrument is to be used amidst the distracting noises of traffic. The end blocks are set as near to the sides of the road as practicable, in order to permit measurements being taken across almost the entire width of the road. Should longitudinal cracks develop through the sections measured, the readings so taken will be rendered useless; and in order to eliminate this difficulty, sufficient plugs must be set to permit obtaining readings at uncracked sections.

Wear measurements of this kind taken of the actual road surface should prove of great future value if the traffic conditions and the physical characteristics of the concrete materials likewise are known, and should help to decide present moot questions regarding concrete roads and road materials. Not only may concrete surfaces be measured for wear in this manner, but the wear or vertical movement of other kinds of road surfaces may likewise be determined by the use of this instrument.

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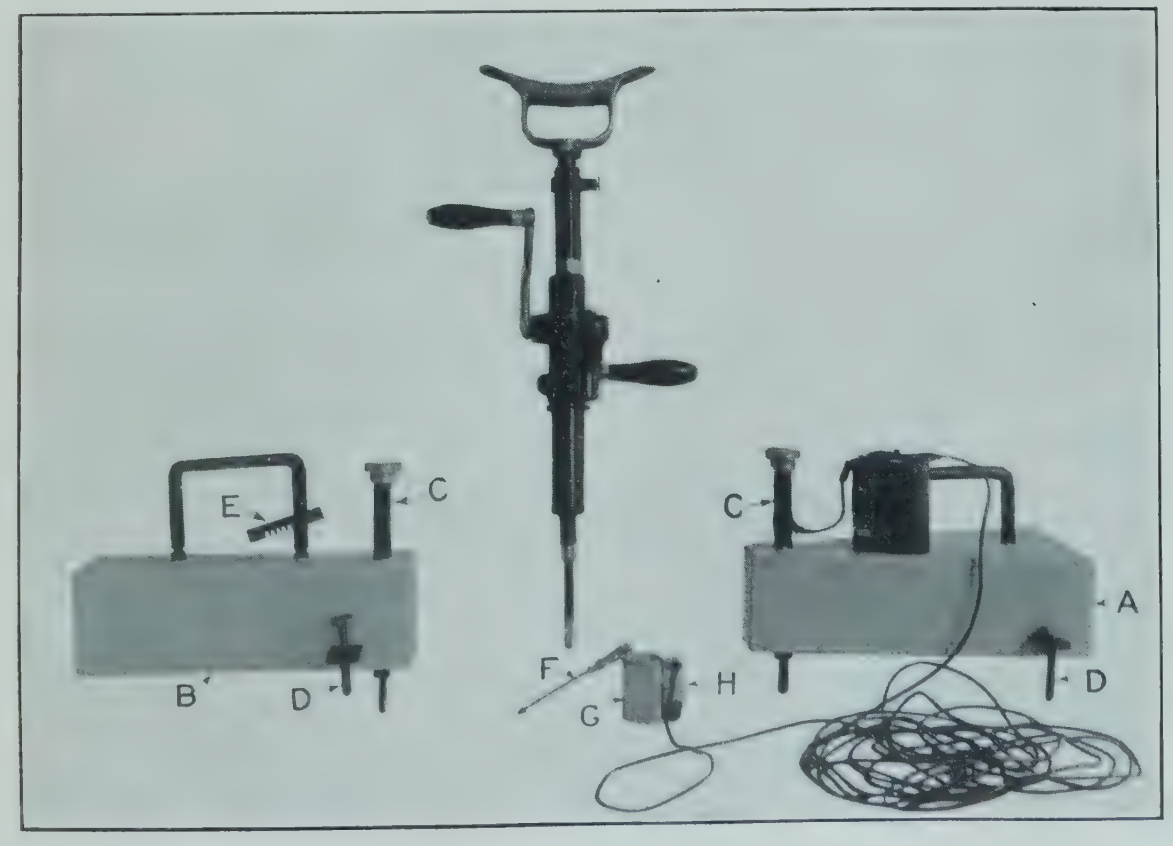

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., FEBRUARY 21, 1916 NO. 21

MORPHOLOGY AND BIOLOGY OF THE GREEN APPLE APHIS

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INTRODUCTION

Owing to the abundance of the green apple aphis (*Aphis pomi* De Geer) at all times in most apple-growing regions and to the serious outbreaks of the species at different places and in different seasons, the writers were instructed to make a careful study of its life history. It was thought best to study the embryology of the insect in order, if possible, to explain the high mortality of the eggs in certain cases, their wintering condition, and, among other things, the most suitable time to attempt their destruction. Eggs were therefore taken during the winter of 1913–14 and again during that of 1914–15. With the opening of the season of 1914 generation experiments were begun at the deciduous fruit insect laboratory at Vienna, Va., and carried throughout the summer, fall, and early winter until the last sexes and eggs of the year were obtained. The material obtained from these experiments and the eggs in hand were studied and the manuscript prepared for publication during the winter of 1914–15.

During the summer the writers were assisted by Miss Dorothy Walton, and for three months by Miss Meta Neuman. These young ladies prepared the mounts of much of the summer material.

NAME OF THE SPECIES

The green apple aphis was first described by De Geer in 1773 (1, p. 53)[1] as follows:

> Aphis (pomi) flavo-virides, corniculis longioribus, pedibus antennisque nigrescentibus, Pomi.

After giving this brief description De Geer enters upon a discussion of the insect, describing the different forms and giving interesting observations on the life history. For so early an account this is a very complete one and is much more valuable than many of those of more recent date.

In 1775 Fabricius (2, p. 737, no. 19) redescribed the species as follows:

> A. Pyri, mali.
> Habitat sub pyri mali foliis.
> Corpus viride, antennis pedibusque fuscis. Abdomen nec marginatum, nec plicatum. Anus terminator stylo nigro. Corniculi cylindrici, nigri. Variat corpore toto rufescente, pedibus fuscis et interdum pedibus lividis, geniculis fuscis.

This name, *Aphis mali* Fab., was that by which the insect was commonly known until recent years. There seems, however, little reason for having adopted it, as Fabricius himself in 1794 (3, p. 216, no. 29) gives De Geer's insect as synonymous with his. He, however, uses his own name "*mali*" for the species and disregards De Geer's "*pomi*" altogether. "*Mali*," then, became the accepted name for the species. Unfortunately in this country the name "*mali*" was for many years applied to an entirely different species, now known as "*avenae* Fab.," under the impression that it was the apple insect of Fabricius. This error was first introduced into the literature of this country by Fitch (5, p. 65), and the same author later (6, p. 753–764; repr. p. 49–60) gave a very good description of *avenae*, under the name "*mali*." In this, however, he was only following European entomologists, such as Walker (4, p. 269), who used the name "*mali*" for an entirely distinct aphis.

Later writers followed in the same path, some, such as Buckton (7, p. 44, pl. 50), even confusing several species under the name. Sanderson (10, p. 191) used the name "*padi*" for this species in 1901. In more recent years De Geer's name has been given preference, and in this country the descriptions of Smith (9) and Sanderson (11, p. 130) have fixed the species to which it should be applied. The insect herein discussed must then be known as "*Aphis pomi* De Geer."

[1] Reference is made by number to "Literature cited," p. 992–993.

HISTORY AND DISTRIBUTION

Apparently the earliest record of the green apple aphis is the description by De Geer (1, p. 53), who states, in connection with this description, that he made rather extended observations of the species during the autumn of 1746. He also states that the insects were very abundant on the apple (*Malus* spp.) and often killed young trees. De Geer's observations were made in Sweden. Since the original description, many other European records have been made, and the species is now known to occur in every country of Europe and at least as far east as Turkestan in Asia. Many writers have reported it as being very injurious, particularly to young trees.

The unfortunate confusion of names makes it impossible to determine to which species the earlier records in this country really pertain. By previous writers *pomi* has been considered of much more recent occurrence in this country than the other apple species, *avenae*. This opinion, however, is not well founded. Although the descriptions given by Fitch (5, p. 65; 6, p. 753–764; repr. p. 49–60) prove that he considered *avenae* to be the true *mali*, an examination of the material from the Fitch collection shows that part of his insects were *avenae* and part of them *pomi*, even as they might be collected to-day by one not knowing the differences between the species. The specimens of *pomi* are marked "showing variations," which would indicate that, although Fitch noted the differences, he did not consider them of specific value. This shows *pomi* to have been located in this country nearly as early as we have any definite records. It was taken in Washington State in 1883 and in the District of Columbia in the same year. Williams collected it in St. Louis in 1894, and in all probability the forms referred to as *mali* by Cowen in 1895, in the bulletin by Gillette and Baker (8, p. 120) were *pomi*, since he observed both winged and wingless insects on the apple on August 23. It was present in Illinois in 1897, and no doubt was well distributed over the country much earlier than we have heretofore supposed.

In 1900 Smith (9) published a life history of this species. His first definite observations were made in 1897, and he first separated the species from the *mali* of American authors. In 1902 Sanderson (11, p. 130) published life-history notes on the species under the name "*pomi* De Geer."

It is known that this species occurs throughout the country wherever apples are grown. The accompanying map (fig. 1) merely shows definite localities from which we have records of the insect. It would indicate that the species is most abundant in the East. This, however, is not the case, since various observers in the West record it as occurring throughout their States. It appears to be particularly abundant in Colorado and the neighboring States.

Aphis pomi also occurs in Canada, being found from Nova Scotia to British Columbia. It has recently been recorded in the Kootenai and Okanagan districts of the latter Province.

Outside of Europe and North America few records of the species occur. It is present in Japan (18) and Dewar (12, p. 12) records it from Orange Free State.

It is rather remarkable that this aphis has not become even more widely spread, since it is typically a nursery species and in the egg state is easily transported on nursery stock.

Both in this country and in Europe *Aphis pomi* is usually abundant and particularly injurious at irregular intervals. Thus, in 1911 a severe outbreak occurred in Virginia, while in 1912 the species was very abun-

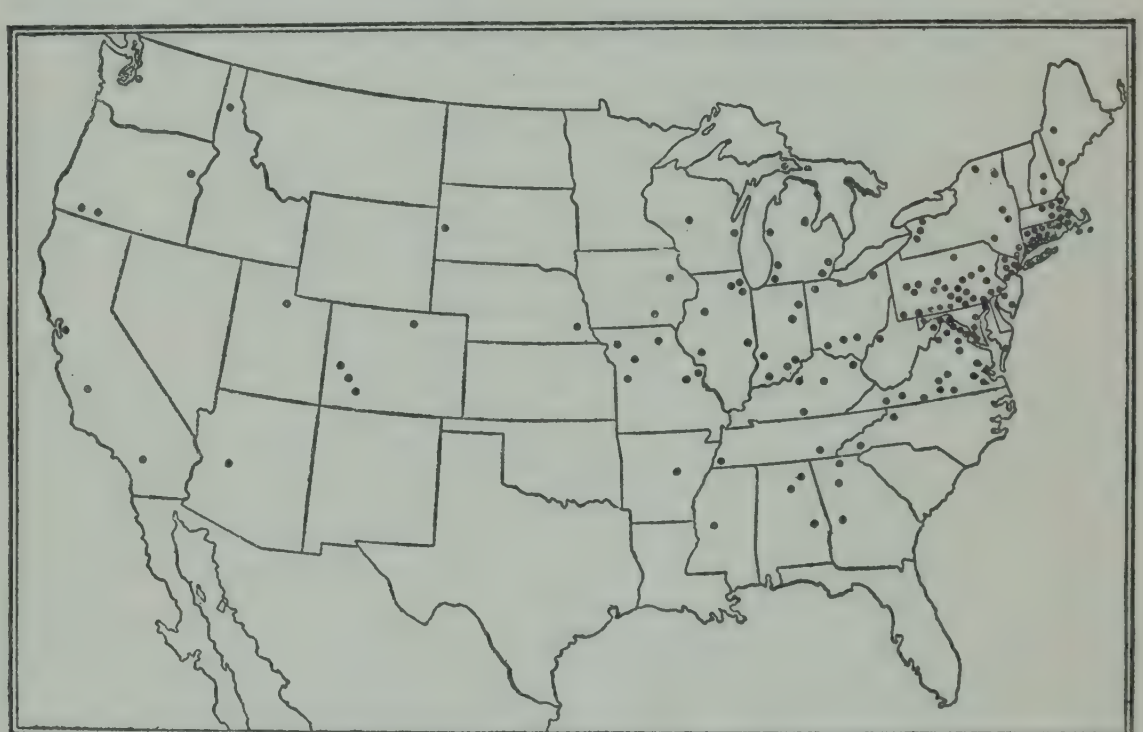

FIG. 1.—Map showing the localities in the United States from which the Bureau of Entomology has actual records of the green apple aphis (*Aphis pomi*).

dant in New England and New York. Similar phenomena have been noted from Russia. In some portions of this country, however, it seems to be always present and injurious. Gillette and Taylor (14) state that in Colorado "*A. pomi* is one of our very worst orchard enemies."

METHODS OF STUDY

EXPERIMENTS.—In initiating the experiments on which the following paper is based, twigs which bore eggs were collected at the time the eggs were beginning to hatch. These were kept under close observation. As soon as an egg hatched, the young stem mother was transferred to another twig kept in a vial of water. Although fairly satisfactory at first, this method of handling the food soon proved to be undesirable. Therefore there were substituted, first, dormant seedlings which had

been kept in a cellar all winter, and later young green apple seedlings grown in pots. In handling the dormant stock the tops were cut off, leaving a stem of 4 or 5 inches, and growth was started by keeping the roots in water for 8 or 10 days before planting.

The plants were covered by lantern-globe cages—inverted lantern globes with cheesecloth fastened over the bottom by a rubber band.

After the first two weeks all work was carried on in an insectary all four sides of which were made of fly screen. This duplicated normal conditions very closely, except that in most cases the direct rays of the sun could not reach the plants during the middle of the day.

In the actual handling of the insects it was found that it was much better to transfer adults than young, as this transfer of adults could be accomplished much more quickly and with greater safety, there being less danger of breaking the beak of the mature insects. Consequently several generations were reared, one after another, on one plant. This was also of great advantage in studying the effect of a prolonged use of good or poor food.

The usual custom in rearing aphides appears to be to raise the first born from the first born and the last from the last throughout the season. Since it was desired to raise young from both wingless and winged mothers in every case, this method proved to be impracticable. Moreover, the opinion was held—an opinion which has been confirmed by the past season's work—that the thorough study of a species can not be accomplished by such methods, because too few insects are reared. Consequently, as many insects as possible were carried to maturity, the number varying between a few to 60 or more for each experiment. The winged forms were transferred to new plants as pupæ. The wingless form was reared to maturity, and then all but from one to three insects were removed, these few being allowed to reproduce. All molts and specimens of insects from each generation were mounted for further study. At first each individual molt was mounted on a separate slide, but later, as their number grew into the thousands, this was impossible and a series of molts was placed on each slide. The total number of experiments conducted during the season was 1,720, with an approximate total of 15,000 insects. These insects, together with their molts, thus gave us for study nearly 75,000 individual forms of known lineage. The study of these forms has been tedious, but it has been a valuable adjunct to the actual breeding, furnishing many data which would otherwise have been unavailable.

It should be understood that, while the method above outlined was followed as closely as possible, it could not, from local causes, be applied in every case. However, it has been found to be very satisfactory and is believed to be a more efficient method for a thorough scientific study of the life history of aphides than any that has been seen recorded.

TECHNIQUE.—For description, specimens were mounted in balsam in the usual way after having been dehydrated and cleared. Eggs were fixed with acetic-alcohol-sublimate solution, and after washing were preserved in 70 per cent alcohol. Those which had been preserved for some months gave better results on sectioning than did newly fixed material. Clearing was done in cedar oil, and sections from 5 to 10μ in thickness were cut; those 8μ gave the best results. Staining was done with Delafield's hematoxylin, orange G and picric acid, and Mayer's acid hemalum. Borax carmine was used for staining in toto.

THE EGG

DESCRIPTION

Size, 0.572 by 0.281 mm. Form oval, flattened on side next the bark; more or less covered with a glutinous substance which hardens with age. Color, glossy black.

The newly laid egg is not, as has been frequently stated for this species, yellowish green in color. It is a decided light-yellow, with rarely a slight tinge of green. It does, however, become somewhat greener during the change from yellow to black. This change is completed in the shade (insectary conditions) in from one to four days, usually a little over one.

The sterile egg can be easily separated from the fertile in that it is orange in color when laid. In one case such an egg finally turned to "ox blood," but this was the only example out of more than a hundred in which any color change took place before the egg began to shrivel up, at which time it sometimes became orange brown. This shriveling usually took place in about a week or 10 days after deposition.

LOCATION ON TREE

The green apple aphis hibernates only in the egg stage. The eggs are laid in the fall on the smooth twigs, and especially on water sprouts. They are apparently never laid on the trunks of the trees, or even upon the branches. This is to be expected, since the females feed continuously during the oviposition period, and they would be unable to obtain their food through the thick bark (Pl. LXXV, fig. 2).

Unless the eggs are very abundant, they are usually deposited around and under the buds and in wounds in the bark. When abundant, however, they will be found scattered promiscuously over the twigs, and in some cases these will be entirely blackened with them. It is very interesting to note that in the winter of 1914 a careful survey of a large bearing orchard near Vienna, Va., revealed the presence of eggs only on trees in the south to west portion, and they were most abundant in the southwest corner of the orchard. These results were duplicated in an examination of a small orchard of 4-year-old trees on the laboratory grounds. More-

over, in both of these cases, and also in examinations of many isolated trees, the eggs were found to be much more abundant on the southwest sides of the trees.

The eggs adhere so tightly to the bark that great care is needed in removing them, and often this can not be done without breaking them. On downy twigs it is impossible to remove the eggs without also removing some of the hairs which adhere to them. Neither alcohol nor xylol will dissolve the adhesive or free these hairs from the egg.

EMBRYOLOGY

GENERAL EMBRYOLOGY.—The substance of the unfertilized egg is very clearly divided into two areas. The first, comprising nearly all the space included within the vitelline membrane, is filled with the food yolk, which consists of homogeneous granules enmeshed in a fine network of protoplasm. The second area, filled with smaller granules, which the writers are calling the "ovarian yolk," following Webster and Phillips (17, p.95), is rather spherical in shape and lies at the posterior pole. Surrounding these two bodies is a very narrow layer of peripheral protoplasm, the periplasm or "*Keimhaut blastem*" in which the blastoderm will form later. The egg is included within two envelopes, the vitelline membrane and the chorion.

At the time of deposition the fertilized egg appears like the sterile egg. In a very short time, however, the production of cleavage cells commences, and the formation of the blastoderm is initiated. This begins at the anterior pole and progresses most rapidly in that region, but in a short time covers the entire yolk, with the exception of the posterior end, where it lies in contact with the ovarian yolk. A portion of the cleavage cells do not migrate to the periphery, but remain in the yolk to become yolk cells.

Invagination commences by a thickening of the blastoderm in its area of contact with the ovarian yolk, brought about by the division of the blastoderm cells along this area.

At the end of about five days the germ band attains a condition in which it rests or hibernates till early spring. In this resting stage the embryo occupies a position in the center of the egg, with its cephalic portion directed toward the posterior pole. The posterior half of the abdominal region is flexed dorsad in such a manner as to include the ovarian yolk. Segmentation is well advanced, and the formation of the appendages has begun. The stomatodeum and proctodeum are present, while the formation of the mesenteron has begun. The genital rudiments are separated into two groups, although the ovarian yolk is not yet divided. At the posterior pole lies an organ composed of a single layer of cells surrounding a pear-shaped orange body without structural characters. This has been designated by Webster and Phillips (17, p. 98) as the "polar organ."

Development is resumed in the late winter or early spring (March 12 to 15, during 1914 and 1915, at Vienna, Va.). Growth is not resumed uniformly, even in a group of eggs on a single twig, some starting two or three days before the majority and a few not beginning to grow till nearly the end of March. This renewed development is accompanied by a movement of the embryo through the yolk toward the posterior pole till that portion of the amnion which lies above the head comes in contact with the serosa at its junction with the polar organ. The two envelopes then rupture at this point and the embryo revolves about its transverse axis to its definitive position.

From this time on development is rapid. The serosa contracts, and is invaginated and absorbed. The appendages are completed, the development of the digestive tract is consummated; nervous and muscular systems are perfected. Within a period of from five days to two weeks, depending apparently entirely upon temperature conditions, the insect is ready to hatch.

OVARIAN YOLK.—At the posterior pole of the egg there is situated an almost spherical, dark-staining body. This has been termed the secondary yolk by most writers, but has been designated the "ovarian yolk" by Webster and Phillips (17, p. 95). The writers are unable to follow the formation of this body, as no egg material earlier than those eggs deposited by the female was preserved. Tannreuther (13) studied its formation in *Melanoxantherium salicis* L. He states that it is formed from the follicular nuclei of the oviduct wall, these dividing to form small vesicles which later unite and form common spherical masses. In the writer's earliest fertilized material (fertilized less than 24 hours) the ovarian yolk consists of a densely granular, almost spherical mass containing a number of large cells (Pl. LXVIII, fig. 7) which would correspond fairly well to the figures given by Tannreuther. At this time (Pl. LXVIII, fig. 1) the writers are unable to observe any cleavage cells within the body of the yolk, although there are at the anterior pole a number of dark-staining bodies well separated, but forming a dome-shaped structure conforming to the shape of the anterior part of the egg.

One thing is worthy of note in this connection. In unfertilized eggs, ranging in age from a few hours to 11 days, the ovarian yolk is a uniform, finely granular mass (Pl. LXVIII, fig. 3) without any of the large cells met with even in our earliest fertilized material. This leads to the belief that these bodies are associated with and appear only in connection with the beginning of growth. At the time the blastoderm is completely formed these bodies are present within the ovarian yolk and are surrounded by darker staining areas (Pl. LXVIII, fig. 2.) When the blastoderm is completely formed it covers the entire surface of the egg with the exception of the ovarian yolk, and invagination takes place about this yolk. (A single yolk cell is shown in Plate LXVIII, figure 6.)

It is thus carried to the interior of the egg with the developing germ band (Pl. LXIX, fig. 1). As the embryo develops, the ovarian yolk remains in connection with its posterior extremity, enlarges, and when this extremity becomes recurved, the yolk may be seen as a large, somewhat dumb-bell-shaped mass lying within the curve. At this time the large, deeply staining cells which form the end chambers of the ovaries are distinctly visible at its extremities. The remainder is a finely granular mass very similar in texture to that of the original ovarian yolk (Pl. LXIX, fig. 2). At a slightly later period the mass of the ovarian yolk becomes somewhat more enlarged in the heads of the dumb-bell at the expense of the "grip," and the end chambers are already forming (Pl. LXX, fig. 1). After the revolution of the embryo, the two heads of the dumb-bell-shaped yolk become separated, and it is henceforth represented by two large, slightly elongated masses, one on either side of the ventral portion of the body, the end chambers distinctly formed, and those on each side connected with one granular body of this ovarian yolk (Pl. LXX, fig. 2). In embryos almost ready to hatch, these two large granular bodies are still present, although more elongate than in the earlier stages. Some of the first egg chambers are now formed, and eggs may be noted within. The remainder of the reproductive organs are not yet developed (Pl. LXXI, fig. 1).

In the first instar of the stem mother these elongate granular bodies are still present. Webster and Phillips (17, p. 99) state that a group of cells which ultimately give rise to the generative organs separate off from the mesoderm during their "stage 6." The results of the present writers do not uphold this view. It seems more probable that these cells develop in the ovarian yolk, possibly from migrants, in the very early stages of growth, and that they are carried to the interior with this yolk at the time of invagination; that they here form two groups, one on either side of the ovarian yolk, which ultimately divides; and that these two masses of the ovarian yolk remain throughout embryonic development and assist in the formation of the reproductive system.

Polar organ.—Upon invagination the germ band leaves behind it, at the posterior pole of the egg, a group of large nucleated cells. This cell group has been recorded by Webster and Phillips (17, p. 98) as occurring in *Toxoptera graminum*, and was designated in their paper as the "polar organ." The writers have been unable to find any other reference in literature to the occurrence of such a body, either in the eggs of Aphididae or in those of any other insects.

The writers have not observed the genesis of this organ, but by the time the embryo has attained its "resting stage" it consists of a single layer of elongate cells surrounding a pear-shaped lumen (Pl. LXVIII, fig. 4). A large nucleus is present in the outer portion of each cell.

The lumen of this organ is occupied by a structureless yellow or orange-colored substance which extends by means of an elongated neck through an aperture in the chorion, thus opening upon the surface of the egg.

Webster and Phillips state that the yellow matter appears like a liquid. In *A. pomi* and in *A. avenae*, in which the organ is also present, it has more the appearance of a wax. Certainly it has a definite form which it maintains even when the surrounding cells are removed from it. The material is not affected by alcohol, xylol, or chloroform.

With the migration of the embryo to the surface and its revolution the cells of the polar organ are withdrawn, leaving the yellow body unchanged in form and still attached to the chorion. In one specimen which was in the late stages of development the yellow body was found inclosed by the anal portion of the embryo. Usually, however, it appears never to come in contact with the embryo; and when the latter hatches, it is left behind in the eggshell. The writers have been unable to find anything resembling it in any of the newly emerged insects.

DORSAL BODY.—With the resumption by the embryo of activities in the spring a change takes place in the cells of the polar organ. These flatten out, drawing away from the yellow mass as if the serosa were exerting an upward pull on them from all sides (Pl. LXVIII, fig. 5). Through the migration of the embryo the amnion finally comes in contact with the serosa at a point where the latter joins the cells of the polar organ, and both amnion and serosa rupture at this point.

As the embryo revolves, the serosa contracts until it lies as a thickened plate, the dorsal plate, near the anterior pole of the egg. In fact, in some cases the thickening takes place directly at the anterior pole, the plate moving later somewhat toward the posterior. During this contraction of the serosa it draws the cells of the polar organ after it, so that when the dorsal plate is formed, these lie as an irregular mass just posterior to the serosal cells (Pl. LXXI, fig. 2).

After the formation of the dorsal plate has been accomplished, this body commences to invaginate at its center, forming a tube which extends into the yolk ventrad, inclining slightly toward the posterior. This tube is formed of both the serosal cells and those which formerly constituted the polar organ. These cells can not now be distinguished from one another (Pl. LXXII, fig. 1).

This dorsal body soon separates itself entirely from the amnion and lies wholly immersed within the yolk in the form of a hollow sphere, one cell in thickness (Pl. LXXII, fig. 2). A little later this sphere breaks up and the cells disintegrate, probably being used as food by the embryo.

RESTING STAGE.—From the standpoint of life history the resting stage is one of the most interesting points in the embryology of this species. The embryo appears to be very seriously affected by changes of temperature at this time, or rather by sudden changes to temperatures

higher than those normally occurring out of doors. Several lots of eggs containing "resting" embryos were taken into the greenhouse at Vienna, Va., during the winter of 1915.[1] The first lot was taken on January 7 and other lots were taken at intervals of from one to two weeks until after growth was resumed. All the eggs in all lots died within two weeks. Over 50 per cent of all eggs placed in the greenhouse after the revolution of the embryo commenced, hatched normally.

It was at first thought that humidity might be a factor in this mortality, but the following experiment eliminated that. A very hairy twig which was well infested with eggs was cut in two. One half was placed in water, just as it was. The hairs acted as a wick, drawing the water to the top of the twig and keeping it and the eggs constantly moist. The base of the other twig was cleaned so that the water could not reach the hairs, and it consequently was dry. Both lots of eggs began to hatch on the same day. Moreover, hatching proceeded a little more rapidly on the dry than on the wet twig. It should be stated that the eggs used in this experiment had resumed growth before being taken into the greenhouse. These results are confirmed by the fact that no difficulty was experienced in hatching eggs taken into warm temperatures after the middle of March.

It will be seen that the temperature effect upon the egg at this period is rather a complicated matter. The activities of the embryo in the spring are apparently initiated by a general rise in temperature above the normal winter average. It seems probable also that these higher average temperatures must continue for some time for this species, since warm weather of two or three days' duration, occurring in January and February, does not appear to induce any growth whatever in the embryo. Certainly there is no appreciable difference between embryos collected just before such a period and those collected after it.

On the other hand, if the temperature affecting the eggs is artificially raised to greenhouse temperature (about 65° F.) at any time before the normal resumption of growth, the embryo dies. It is true that in certain instances some activity is induced, and embryos treated in this manner will be found to have developed somewhat, but in no case in these experiments did the revolution of the embryo occur.

From data of the writers it would seem that the embryos need to pass through a period of cold weather, perhaps even need to be subjected to freezing temperatures. This is indicated by the fact that in eggs laid early in the season the embryos had reached the resting stage and ceased growth for three weeks or a month before later eggs were deposited. Yet these later eggs in their turn developed normally to the resting stage.

The amount of low temperature needed by the insect is very uncertain. As suggested previously, it may be that a single freezing is sufficient, or

[1] The average temperature in the greenhouse was about 65° F.

it is possible that continued cold weather, or a succession of freezings, is essential. In either case it seems probable that the embryo must have experienced a sufficient amount of low temperature long before spring and that it must thereafter continue to remain dormant till the proper average temperatures exist for its renewed activities. If, however, the embryos be subjected to temperatures well above the critical at any period before they have revolved, this change is fatal to them.

What this critical temperature is, can not be determined with any exactness from the data at hand. In 1915, from March 8 to 16, the period during which growth was resumed, the average temperature dropped to 34° F. only once, and it was below 36° only twice in the week. In 1914, however, the averages varied between 18° and 60° during what appears to have been the critical period, although from March 14 to 18, inclusive, it was above 34°. It seems probable that the critical temperature is close to 36°. Apparently, also, this critical temperature, or average temperatures a little above it, must continue for a period of some days, since frequently average temperatures higher than the critical occur for one or sometimes more days in January and February without affecting the insects.

It is interesting to note that eggs of *A. avenae* brought into the greenhouse during the winter hatched normally. Eggs of this species frequently hatch on the trees after warm spells of two or three days' duration in January and February; and while the writers have not as yet made a thorough study of the embryology of this species, yet during the winter they have taken several eggs in which the embryo had revolved.

These observations are of particular interest, since they undoubtedly explain the fact stated by several writers that a very low percentage—about 2 per cent, according to Gillette and Taylor (14, p. 24)—of the eggs of *A. pomi* hatch.

HATCHING.—The first eggs hatched in 1914 about April 8 and the last about April 25. At this time nearly all the buds showed some green and in many cases the tiny leaves were free from the bud scales. Since it is as immature stem mothers that this and corresponding species are usually treated with insecticides, it will be well to include here a comparison of their dates of hatching. In the spring of 1914, at Vienna, *A. avenae* commenced hatching on March 28. *A. malifoliae* and *A. pomi*, however, did not hatch until about April 8. A few eggs of *A. malifoliae* hatched before that date, and this would seem to indicate that the rosy apple aphis is perhaps slightly earlier than the green aphis. For all practical purposes, however, their hatching dates are the same, while that of *A. avenae* is very much earlier.

The young stem mother emerges from the egg head foremost, and the latter is always split evenly over the vertex of the insect. This is accom-

plished by means of a bladelike egg burster, which extends from the region of the trophic tubercle over the vertex and backward on the crown as far as the posterior margin of the eyes (Pl. LXXIII). This egg burster is often armed with one or two toothlike projections on its cutting edge. After the shell has been ruptured, the young, still within the membrane, protrudes for almost its entire length before the membrane ruptures. It is not uncommon to find insects which have reached this stage and died. They stand upward almost out of the shell, but still within the membrane. After the membrane has become ruptured and the insect has emerged, the former position of the egg burster is indicated by a suture-like marking extending over the vertex and crown and separating the two halves of the dark-colored cap met with in the stem mother of this species.

PLAN OF DESCRIPTIONS

It has been found by the study of the different instars that the easiest method for separating them is by the character of the antennæ. By measurements of these organs it is possible to determine immediately the instar of the form examined. In describing the different stages, therefore, in the earlier instars, measurements of the antennæ only are given, and these are followed by a complete description of the adult form. In the third instar of the summer forms those insects destined to become pupæ can be distinguished from those destined to become wingless only by the presence of the beginning of the wing pads. The measurements for both are the same. For the first two instars, therefore, only one description is given. The pupæ of the intermediate and that of the winged form are the same in every respect, and, therefore, only one description is given for these forms.

It is often important to know, immediately on their hatching from the egg, to what species apple aphides belong. We give here, therefore, measurements of the antennæ of the first-stage stem mothers of the more common apple-infesting species which are likely to be confused—viz, *pomi*, the green apple aphis, *avenae* (Pl. LXXIV, fig. 15, 18), the apple-grain aphis, and *malifoliae* (Pl. LXXIV, fig. 17), the rosy apple aphis. The adult stem mothers of these species could hardly be confused, on account of their different color characters, but the newly hatched insects are most easily and definitely separated by an examination of the antennæ.

The relative lengths of the proximal and distal portions of the fourth antennal segment in the different species are given in Table I, and an examination of these figures will enable one to separate the species easily.

TABLE I.—*Lengths of third antennal segment and of proximal and distal portions of the fourth segment in Aphis pomi, A. avenae, and A. malifoliae*

Species.	Segment III.	Segment IV base.	Unguis IV.
	Mm.	*Mm.*	*Mm.*
Aphis pomi	0.08 to 0.09	0.048 to 0.056	0.048 to 0.064
Aphis avenae	.08 to .096	.03 to .048	.08 to .104
Aphis malifoliae	.128	.048	.048 to .16

It will be noted that *A. pomi* has a much shorter unguis than either of the other species and this at once distinguishes it.

STEM MOTHER

DESCRIPTION

FIRST INSTAR.—Morphological characters: Antennal segments (Pl. LXXIV, fig. 16) as follows: I, 0.032 mm.; II, 0.032 mm.; III, 0.08 to 0.09 mm.; IV, base 0.048 to 0.056 mm., unguis 0.048 to 0.064 mm.; segments III and IV imbricated and covered with a few stout spines, III armed with a distal sensorium, and IV with a sensory group at the base of the unguis composed of one large sensorium and several small ones. Eyes with 8 to 10 facets. Rostrum long. Cornicles short, thick, and rounded at the distal extremities. Cauda and anal plate rounded and densely setose. Legs with spinelike hairs.

Color characters: Body dark green (sometimes very dark) with appendages dusky; crown dusky to black with a median longitudinal uncolored suture-like stripe. Entire insect often slightly pruinose.

SECOND INSTAR.—Morphological characters: Antennal segments as follows: I, 0.032 mm.; II, 0.033 mm.; III, 0.08 mm.; IV, 0.048 to 0.064 mm.; V, base 0.048 to 0.064 mm, unguis 0.048 to 0.088 mm., usually about 0.075 mm.; segments III to V imbricated and with a few stout, spinelike hairs, IV with a distal sensorium similar to that on III of first instar, and V with the usual sensory group. Eyes with about 10 facets. Rostrum comparatively shorter than in first instar. Cornicles short and thick and rounded distad. Cauda and anal plate rounded and setose. Legs more slender than in preceding instar.

Color characters: Similar to the first instar, but lighter in color.

THIRD INSTAR.—Morphological characters: Antennæ much more slender than in the previous instars, with lengths as follows: I, 0.045 mm.; II, 0.042 to 0.045 mm.; III, 0.112 to 0.144 mm.; IV, 0.064 to 0.096 mm.; V, base 0.064 to 0.08 mm., unguis 0.088 to 0.112 mm., usually about 0.1 mm.; sensory characters as in previous instars. Eyes with over 20 facets; cornicles more elongate than in the other instars and not so rounded distad; cauda and anal plate rounded and setose.

Color characters: Similar to those of the previous instar.

FOURTH INSTAR.—Morphological characters: Antennæ fairly long and slender, with lengths as follows: I, 0.053 mm.; II, 0.048 mm.; III, 0.192 to 0.224 mm.; IV, 0.088 to 0.128 mm.; V, base 0.08 to 0.096 mm., unguis 0.112 to 0.128 mm.; segments III to V strongly imbricated, sensory characters as in other instars. Eyes with about 40 facets. Cornicles 0.153 mm. in length and imbricated. Cauda somewhat conical. Legs slender, tibiæ somewhat curved, 0.571 mm. long.

Color characters: Approaching those of the adult form.

FIFTH INSTAR (ADULT).—Morphological characters: Antennæ (Pl. LXXIV, fig. 5) rather long and slender, with lengths as follows: I, 0.06 mm.; II, 0.056 mm.; III,

0.296 to 0.416 mm.; IV, 0.16 to 0.192 mm.; V, base 0.096 to 0.12 mm., unguis 0.16 to 0.184 mm.; segments III to V imbricated and armed with several prominent spinelike hairs, segment IV with a distal sensorium, and V with the usual group. Eyes prominent and with very many facets, ocular tubercles distinct; lateral thoracic tubercles prominent; abdominal tubercles not so prominent. Cornicles cylindric, tapering, imbricated and sometimes slightly flanged, 0.288 to 0.368 mm. in length. Cauda narrow, conical, or very slightly constricted toward its middle, densely setose and armed with a few long curved hairs. Anal plate rounded, setose, and hairy. Legs slender, hind tibiæ 0.752 to 0.88 mm. long. Body quite globose, more so than that of summer form. Length, 1.92 mm.; width, 1.25 mm.

Color characters: General color green, somewhat darker than the summer forms; vertex and crown black; cornicles, cauda, and anal plates black, as are also the tarsi and the distal extremities of the tibiæ, femora, and labium; eyes deep brown. The entire insect is sometimes covered with a bloom.

LENGTH OF NYMPHAL LIFE

The newly hatched stem mothers spend the first day wandering about over the twig on which they were born, doing little or no feeding. They finally settle on the tiny leaves or in some instances on buds in which the green of the leaves barely shows. From this time on they feed almost continuously, seldom changing their positions unless the food is very poor. In that case they may wander about on the twigs. Such insects, however, are very likely not to settle permanently nor to live to reproduce.

The duration of the first instar of stem mothers averages from 4 to 5 days; that of the next three, 6 days, the time being equally divided among the three. The total nymphal life thus averages from 10 to 11 days. That the first instar is longer than the three others, and also longer than the first instar of later generations, is due to the fact that the young stem mother loses one or more days in searching for suitable food. Prolonged cold spells would undoubtedly retard this development somewhat, but the insects can withstand short spells of severe weather with little or no apparent effect. Poor food conditions would probably check their growth also, but this factor is negligible, since the same conditions which induce hatching also cause the buds to burst, so that the food is practically always ready for the insects. Moreover, as stated previously, insects which fail to locate good food, and wander about, seldom reach maturity.

REPRODUCTION

The stem mothers begin to reproduce in about 24 hours after becoming adult. In the experiments of the writers the greatest number of young produced by one stem mother was 42, during a period of 10 days. In most of the species which have been carefully studied the average reproduction by stem mothers is greater than that by any of the succeeding generations. Considerable difficulty was experienced in handling this

form, many of the aphides leaving the plants and dying before the reproductive period was finished. Consequently 42 young is probably below the average under natural conditions.

The young are produced in groups of varying numbers and with unequal periods between the groups. In a general way an adult will produce a group one day and rest the next, but often the rest period will be longer and sometimes shorter. Individual mothers vary greatly in their rate of reproduction from the average rate. Some stem mothers ceased to reproduce for 2 or 3 days between some groups, while others never rested long. The greatest number of young produced in 24 hours was 9, one insect producing this number at two different times. In 4 days 22 were produced by one mother. The average daily production was 4.2.

LONGEVITY

The greatest length of life observed was 20 days. This is undoubtedly much below the true maximum and probably somewhat below the average. In the case recorded the insect produced young up to the last day.

The first stem mothers were observed on April 8 and the last May 6. Under natural conditions this period may perhaps be a little longer.

SUMMER FORMS

NUMBER OF FORMS

Beginning with the second generation and continuing until the sexes were produced, the writers found three adult forms to be present. The most abundant form was the wingless viviparous female. This occurred in every generation, and, with the exception of the second, always outnumbered the other forms present. It would often appear, in definite lines of descent, for several generations without being accompanied by winged insects. In fact, one purely wingless line was carried from the stem mother to the sexes, although in this case winged forms sometimes occurred as sisters or cousins.

On the other hand, the winged form was much more abundant than seems to be the case in most of the other species which have been studied. Winged insects were obtained in every generation from the second to the sixteenth, inclusive, although they became rare after the thirteenth generation.

The third form, the intermediate, occurred in 16 experiments, the first occurrence being in the third generation and the last in the twelfth.

In all, there were from 7 to 17 generations of the summer forms, the number depending upon whether the first or the last young were taken as mothers in each generation. In view of the fact that we have found winged forms to occur so commonly, it is difficult to understand how Smith (9) could have come to the conclusion that no winged insects

occurred after the third generation, an error in which he has been followed by many writers. He also states that only seven generations of the summer forms occur, another error which has been frequently quoted.

WINGLESS VIVIPAROUS FEMALE (PL. LXVII, FIG. 5)

DESCRIPTION

First instar.—Morphological characters: Antennæ (Pl. LXXIV, fig. 4) as follows: I, 0.034 mm.; II, 0.036 mm.; III, 0.120 to 0.144 mm.; IV, base 0.064 mm., unguis 0.112 to 0.128 mm.; segments III and IV imbricated and armed with a few spinelike hairs, III with a distal sensorium, and IV with the usual sensory group at base of unguis. Eyes with 12 to 14 facets; cornicles short, thick and rounded distad; legs thick, hind tibiæ 0.239 mm. long.

Color characters: Color very variable from a light or dark green to yellowish. In some cases the insects are a golden yellow; the normal color is a medium green, never, however, as dark as the stem mother. Appendages dusky.

Second instar.—Morphological characters: Antennæ (Pl. LXXIV, fig. 3) more slender than those of the other instars.; lengths as follows: I, 0.045 mm.; II, 0.046 mm.; III, 0.112 to 0.152 mm.; IV, 0.08 to 0.096 mm.; V, base 0.056 to 0.08 mm., unguis 0.144 to 0.176 mm.; segments III to V imbricated and with a few spines, IV with distal sensorium similar to that on III of first instar, and VI with the usual group. Eyes with 28 to 30 facets. Cornicles rounded at the distal extremity, thick and imbricated. Legs stout and covered with spinelike hairs, hind tibiæ 0.320 to 0.384 mm. in length. Cauda and anal plate setose, cauda somewhat conical.

Color characters: Similar to those of first instar.

Third instar.—Morphological characters: Antennæ (Pl. LXXIV, fig. 2) rather long and slender; lengths as follows: I, 0.048 mm.; II, 0.051 mm.; III, 0.192 to 0.248 mm.; IV, 0.112 to 0.144 mm.; V, base 0.08 to 0.096 mm., unguis 0.2 to 0.232 mm.; segments III to V imbricated and bearing a few spines, the base of V strongly but regularly imbricated but the unguis quite regularly, so giving the appearance of almost complete rings; sensoria as in previous instar. Eyes with 38 to 40 facets. Cornicles slightly rounded at distal extremity, but not nearly as much as in previous instars, length about 0.188 mm. Legs more slender than in previous instars, hind tibiæ 0.448 to 0.054 mm. long. Cauda and anal plate setose, cauda bluntly conical.

Color characters: Similar to those of first instar.

Fourth instar.—Morphological characters: Antennæ (Pl. LXXIV, fig. 10) long and slender; lengths as follows: I, 0.62 mm.; II, 0.06 mm.; III, 0.144 to 0.192 mm.; IV, 0.134 to 0.176 mm.; IV, 0.152 to 0.192 mm.; VI, base 0.088 to 0.112 mm., unguis 0.248 to 0.304 mm.; segments III to VI distinctly imbricated and armed with a few prominent hairs, segment V with a distal sensorium (the original III of first instar now represents III, IV, and V). Eyes with about 58 facets. Cornicles rather slender, compared with the earlier ones, cylindric, imbricated, and about 0.264 mm. long. Hind tibiæ 0.672 mm. long. Cauda and anal plate setose, anal plate rounded, cauda bluntly conical.

Color characters: Similar to those of first instar. The appendages are here partly turned to the black color met in the adult form. The cornicles blacken from the distal extremity proximad.

Fifth instar (adult).—Morphological characters: Antennæ (Pl. LXXIV, fig. 1) long and slender compared with the early instars, but short compared with the body; lengths as follows: I, 0.064 mm.; II, 0.063 mm.; III, 0.224 to 0.320 mm.; IV, 0.176 to 0.240 mm.; V, 0.176 to 0.232 mm.; VI, base 0.104 to 0.128 mm., unguis 0.28 to 0.32 mm.; segments III to VI imbricated and with a few stout hairs; sensoria as in fourth instar. Vertex slightly rounded. Prothorax with a prominent tubercle on each side.

Abdomen with five distinct tubercles on each side, the one pair caudad of the cornicles and the most cephalic pair about equal in size and larger than the three median pairs. Cornicles (Pl. LXXIV, fig. 12) subcylindric, largest at the base, tapering slightly distad, slightly flanged at the tip and strongly imbricated, 0.398 mm. in length. Anal plate rounded, setose, and armed with about a dozen long curved hairs. Cauda (Pl. LXXIV, fig. 19) elongate, rounded distad, sometimes slightly constricted in the middle, setose, and armed on each side with about five long, curved hairs; length, about 0.176 mm. Legs slender, hairy, particularly the tibiæ; length of hind tibiæ, 0.837 mm.; hind tarsi, 0.112 mm.; length of insect from vertex to tip of cauda, 2.56 mm.

Color characters: General color very variable, from a light green to a very dark green. Head orange-yellow, sometimes with a purplish cast. This orange-yellow head is in many specimens much more pronounced than in others. Thorax similar to the head in color, shading off into yellowish green at the abdomen. Both head and thorax covered with a slight bloom. Abdomen light green. Antennæ yellowish, dark toward the tip; tarsi, cornicles, cauda, anal plate, distal extremities of femora, and proximal and distal extremities of the tibiæ black. Labium tipped with black. In specimens which have not been well supplied with food and which consequently are much stunted in growth, the colors are much deeper, the green being very dark over the entire body, whereas in well-fed, large specimens the color is light green. Late fall specimens which are exposed to low temperatures have a brownish cast.

OCCURRENCE

As stated previously, this was by far the most common form occurring during the summer. Moreover, in so far as the actual propagation of the species is concerned, it is the only summer form necessary, since we were able, without difficulty, to carry insects from the stem mother to the sexes without the intervention of a single winged individual. For the spread and consequently the greatest development of the species, winged summer forms seem necessary, since at the present time it has no other natural mode of becoming wholly disseminated. In nurseries the wingless insects may travel from tree to tree in the rows, and trees bearing eggs may be shipped to different parts of the world. Such dissemination, however, would be of little avail to a purely wingless species, as compared to one containing winged forms, since its attack thereafter would be confined to trees on which it was shipped, or at most to a few surrounding trees.

LENGTH OF NYMPHAL LIFE

The average duration of the nymphal period in this form was 7 to 8 days, the time being equally divided between the four stages. During the hot weather occurring in the last of June and first of July this period was shortened to 6 days, and in one instance an insect commenced reproduction when only 5 days old. On the other hand, with the beginning of cooler weather in the late summer the period exceeded this average. About September 1 the time occupied by the nymphal stages was from 8 to 9 days. This period gradually increased in length till the last of September, at which time it covered 11 days. During the month of

September the temperature dropped below 50° F. several times, reaching 37° in one instance. These extreme temperatures were of short duration, however, and the mean was never below 50°. By the end of October the nymph required 12 to 14 days to attain the mature condition. At times during this month the temperature averaged between 53.5° and 59° for periods of 24 to 36 hours. During such periods very little feeding or growth took place. The insects would stand perfectly motionless. Mechanical stimulus with a needle merely induced slight movements of one or two legs. Moreover, it required considerable time for the insects to recover from such conditions, and often maximum temperatures of 65° to 70° would not cause a resumption of active feeding.

The difficulty of exactly correlating the rate of growth with temperature conditions is greatly increased by the fact that the condition of the food supply was as great or even a greater factor in determining this rate of growth. This factor can only be appreciated, however, in marked cases. Usually the observer is unable to determine which of two plants offers the insects the best food, and consequently is unable to gauge the proper values of the two factors. The effect of the food condition is taken up more fully in another place (p. 983).

REPRODUCTION

As in the stem mothers, the wingless viviparous females begin reproduction about 24 hours after becoming mature. In fact, this condition obtained for all viviparous females, whatever the form.

The average reproduction varies greatly during the season and the writers find that their figures separate into three well-defined groups: First, reproduction by the summer forms born before July 1, and reproducing by July 6; second, reproduction by forms born between July 1 and September 1, beginning to reproduce between July 6 and September 10; third, forms born after September 1, commencing reproduction after September 10. Eighty wingless individuals in the first group produced an average of 55.4 young per insect; 113 wingless individuals in the second group averaged 30.9 young, while in the third group 24 wingless individuals averaged 12.1 young apiece. The last mothers of the season produced only from 1 to 4 or 6 young. The average reproduction per insect per day during the first period was 2.95, during the second 1.92, and during the third 0.83.

For the entire season the average per wingless insect was 37.5, and the daily average was 2.22. The greatest number of young produced by one individual was 133, while the maximum reproduction for one day was 16+, one insect producing 64 young in 4 days.

The rate of reproduction was very irregular. In some cases the majority of young were produced early in the life of the adult. In others comparatively few were produced during the first few days and then large

numbers were brought forth. Some insects bore numerous young daily till death; with others the production decreased gradually to that point; while in a third class the insects lived from 3 to 44 days after reproduction ceased, the longer period occurring in the fall, October and November. During the summer the longest period was 13 days. In one remarkable case an insect born on September 29 produced 10 young in 13 days (October 13 to 26). It then ceased to reproduce till December 5 (40 days), when it bore one young and died.

LONGEVITY

The average total length of life for the entire season was 30.9 days. This average is only for insects which reached maturity. Many died while still nymphs. The greatest length of life attained by one insect during the summer was 48 days. In the fall the average period was longer than in the summer, and one insect lived 68 days.

Wingless viviparous females were present on the trees until within less than a week from the time of the last appearance of oviparous females—i. e., during the fall of 1914 until after November 20. In the cages one insect was alive on December 22.

HARDINESS

A rather interesting note was made during the fall on the effect of low temperature on the activities of this species. On December 22 an examination of about 50 insects, including wingless viviparous females and oviparous females, showed all the insects to be perfectly motionless, except one viviparous female. This insect moved both legs and antennæ when irritated slightly with a camel's-hair brush. The temperature at the time the observations were made was 34° F. and had remained constant for about 2 hours. For the 12 hours previous the temperature had been 30° F. or less. This would indicate that at least in individual cases the developmental or physiological zero for this species is quite low.

WINGED VIVIPAROUS FEMALE (PL. LXVII, FIG. 1)

DESCRIPTION

FIRST, SECOND, AND THIRD INSTARS.—In the first and second instars these insects are identical in form with those producing wingless adults. In the third instar the measurements are the same for those given under third instar wingless female, but beginnings of wing pads are present.

FOURTH INSTAR (PUPA) (PL. LXVII, FIG. 3).—The pupæ producing intermediates and those producing winged forms are identical, as follows:

Morphological characters: Antennæ as follows: I, 0.06 mm.; II, 0.06 mm.; III, 0.176 to 0.256 mm.; IV, 0.128 to 0.176 mm.; V, 0.128 mm.; VI, base 0.80 to 0.112 mm., unguis 0.216 to 0.28 mm.; sensoria, imbrications, etc., as in the wingless form. Vertex rounded, with a slight median indentation. Eyes prominent, with a large number of facets; ocular tubercles distinct and with usually three lenses. Thoracic and abdom-

inal sutures as in the wingless form. Wing pads prominent, extending somewhat caudad of the hind coxæ. Cornicles subcylindric, imbricated, slightly flanged; length, 0.168 to 0.376 mm. Legs slender, hairy, hind tibiæ 0.504 to 0.64 mm. long. Anal plate rounded, setose and armed with hairs. Cauda (Pl. LXXIV, fig. 21) conical, not as in the adult form, setose, and armed with many long, curved hairs. Length from vertex to tip of cauda, about 2.6 mm.

Color characters: General color greenish; head and thorax orange-yellow with a rosy bloom, the reddish appearance of this increasing with age. Abdomen yellow-green. Antennæ yellowish, with the distal segments dusky. Wing pads brown, with black costal margins. Eyes, tip of labium, tarsi, and distal extremities of tibiæ and tarsi black; cauda lighter than abdomen, not black as in adult. Area between cornicles darker green than the rest of the abdomen. In some cases the margins of the thorax are light-straw color, almost white, venter usually lighter than dorsum.

Fifth instar (adult).—There is no distinct spring or fall migrant in this species. All the winged individuals occurring throughout the spring, summer, and fall have the same characters and are identical, except for variations bearing no relation to season.

Morphological characters: Antennæ (Pl. LXXIV, fig. 7) as follows: I, 0.064 mm.; II, 0.063 mm.; III, 0.192 to 0.312 mm.; IV, 0.144 to 0.288 mm.; V, 0.144 to 0.224 mm.; VI, base 0.096 to 0.128 mm., unguis 0.288 to 0.344 mm., segments III to VI imbricated and armed with a few hairs, III with a row of usually 6 circular sensoria (range 4 to 9). These sensoria form an even row along the segment and are of about the same diameter as the segment. They have a distinct double rim. Segment IV often without sensoria, although on some specimens there are as many as 3 on this segment near its distal extremity. Sometimes one antenna has sensoria here and the other none. Segment V with a distal sensorium, and VI with the usual group at the base of the unguis. Vertex slightly rounded, median ocellus protruding, lateral ocelli very close to the compound eyes; these eyes large and showing with distinct tubercles. Thoracic and abdominal tubercles as described for the wingless form. Wings with delicate veins; forewing with the media normally twice branched, but not uncommonly with it only once branched and in rare cases (approaching the intermediate) this represented by one vein only. Cornicles (Pl. LXXIV, fig. 11) subcylindric, tapering toward the tip, imbricated and slightly flanged; length, 0.192 to 0.352 mm. Anal plate rounded, setose, and armed with a number of long, curved hairs. Cauda elongate, slightly constricted in the middle, rounded at the tip, densely setose, and armed on each side with about 5 long, curved hairs. Legs slender; hind tibiæ 0.56 to 0.992 mm. long. Length from vertex to tip of cauda, about 2.5 mm.

Color characters: Head and thorax shining black, sutures yellowish; antennæ straw color at base, dark, almost black distad; eyes black; legs yellowish, with the distal extremities of the femora, the distal and proximal extremities of the tibiæ, and the tarsi black. Abdomen yellow-green, with the margins and a longitudinal median stripe darker green. Cauda and anal plate black. Labrum straw color, with tip dusky or black. Wings hyaline, veins brown, stigma smoky.

Most of the winged forms had the abdomen uniform green, but with the second winged generation another form appeared. The color of this is as follows: Head and thorax black, similar to the first winged generation; veins and stigma dark; abdomen unlike the uniform pea green of the first winged generation, but much darker, with a median longitudinal stripe of still darker green; margin of the abdomen on each side with a row of 5 or 6 dark patches; other characters as in first winged generation.

The color characters of this winged generation may have had something to do with the confusion of *A. pomi* and *A. avenae*, as the color characters of the two are quite similar.

CAUSE OF PRODUCTION

The theory has been frequently advanced that the production of winged forms during the summer is due to a lack of sufficient nourishment for the insects. In some cases the wording of this theory is modified by the statement that winged forms appear on plants which are very heavily infested. The writers' results are a flat contradiction of this theory for this species.

As has been stated previously, in handling the insects the writers always transferred the mothers to new plants, rather than the progeny. In this way several consecutive generations were reared on one plant. Thus the effect of poor or good food would be accentuated. Yet the winged forms were never obtained in series of small, poorly fed insects, but occurred frequently in well-nourished series.

It should be stated that these results are not based on deliberate experiments to obtain data on this point. Notes were made simply because of a very evident abnormality in size and rapidity of development, correlated with a lack or an abundance of food. Later, in studying the notes, it was found that the large, well-fed insects developed rapidly and often produced winged forms, while many of the small, starved aphides produced only wingless progeny. Moreover, none of the plants was heavily infested, so the production of winged aphides can not be correlated with that condition.

In addition to the foregoing data, it was found that those winged insects produced during the summer months showed little or no inclination to leave the plants on which they were produced. This would at once disprove the theory that these winged forms are produced when the insect meets adverse food conditions in order to carry it to better food.

Other writers have maintained that the winged insects were produced as the result of an abundance of certain chemicals in the soil. The writers' work would not certainly contradict this theory. Still, the fact that the soil used was mixed in large batches and that winged forms were produced on some of the plants, while other plants raised in soil from the same batch bore only wingless forms would seem to cast considerable doubt on its truth. It is also very difficult to understand how the occurrence of such a form as an intermediate could be made to conform to this theory.

The writers' results, deduced from very full notes on the life history of this aphis, lead to the belief that much of the evidence given in favor of these theories is based on insufficient data.

It seems much more probable, especially in view of the quite frequent occurrence of such a form as the intermediate, that the production of this winged form during the summer is merely a reversion from the wingless to the more primitive aphis form. As such it is doubtful whether food conditions have anything whatever to do with the matter.

OCCURRENCE

Although, as has already been stated, this form is not necessary for the successful propagation of the species, it occurs quite commonly throughout the greater part of the summer. In the second generation the winged form outnumbers the wingless, although the writers were unable to determine the exact proportion. Thereafter winged insects are always less abundant than wingless.

This form occurred, in the writers' experiments, in every generation from the second to the fifteenth, inclusive. It was of very rare occurrence, however, after the thirteenth generation. In the complete life-history diagram (fig. 4) it occurred 149 times, each occurrence representing a different combination of the two factors, form and generation, among the ancestors.

In the field winged forms were apparently present in small numbers all summer. Definite observations were made on several days during July and August. In all cases migrants were found in every colony of any considerable size.

It is very interesting to note that in only 18 cases were winged forms produced by winged mothers, and in only one case did three winged generations occur in succession.

The last winged insects were born on September 9; none were found after October, either in the experiments or on the trees.

LENGTH OF NYMPHAL LIFE

The immature stages of this form covered a period of two more days than did the same stages of the wingless form. This extra time was occupied in the pupal instar, the three earlier stages requiring the same amount of time as the corresponding stages of the wingless form.

REPRODUCTION

Dividing the season into periods similar to those used in the discussion of the wingless reproduction, the writers obtain the following figures: The average reproduction by 29 winged insects during the first period (to July 1) was 50.1 per mother; that of 25 insects in the second period (July 1 to September 1), 25.4 per mother. Very few winged insects occurred during the third period, and the writers have no complete records of progeny from any individuals. During the first period the average per insect per day was 2.92. During the second period it was 2.04.

The seasonal average production per insect was 39, while the daily average was 2.62. The greatest number produced in one day was 6, and the maximum number of young produced by one individual was 120 (in 21 days). The average length of the reproductive period for the entire season was 20.75 days.

LONGEVITY

The longest total life recorded for an individual of this form was 42 days.

FLIGHT

A large number of migrants of the second generation were reared on some small apple trees in the laboratory. These insects, on becoming adult, were very active, and several hundred were taken on the windows of the room in which they were confined. They were to a marked degree negatively geotropic. This was well illustrated by the fact that as many as 25 could be kept safely in a small open vial by simply holding it upside down. Almost without exception migrants transferred to new plants settled readily and made no attempt to fly farther. They were very likely to fly from the brush, however, during the process of transfer.

In the case of the later winged forms no such tendency toward flight was observed. In no case were winged aphides observed which had left the plants and clustered on the sides and tops of the cages, unless the plants were so nearly dead that the wingless forms also left them. Moreover, no particular caution was necessary in transferring them from one plant to another, since they showed no inclination toward flight. This would seem to indicate that the winged forms of the second generation alone correspond to the spring migrants of species with a definite alternation of hosts.

INTERMEDIATE FORM (PL. LXVII, FIG. 6)

DESCRIPTION

Morphological characters: Antennæ (Pl. LXXIV, fig. 6) as follows: I, 0.064 mm.; II, 0.064 mm.; III, 0.28 to 0.34 mm.; IV, 0.16 to 0.24 mm.; V, 0.144 to 0.208 mm.; VI, base 0.096 to 0.12 mm., unguis 0.176 to 0.328 mm. Antennal segments armed as in wingless individuals, with the exception of segment III, which is armed with unequal sized sensoria, varying from 4 to 6 in number. Vertex rounded; eyes with ocular tubercles present; ocelli absent, even from specimens with nearly half-size wing rudiments. Thorax and abdomen with tubercles as in the wingless form. Thorax not showing the distinct "corseletta" of the winged form, but indicating a series in these forms from the winged to the wingless condition. Wings of winged form represented here by reduced wings of about half the normal size, through gradations in different individuals until mere folds of the skin are seen. Cornicles subcylindric, tapering distad, imbricated, and slightly flanged; length, 0.272 to 0.496 mm. Anal plate rounded, setose, and armed with long hairs. Cauda elongate, slightly constricted in the middle, rounded at the tip, densely setose, and armed with five or six long curved hairs on each side. Leg slender, hairy; hind tibia 0.608 to 0.896 mm. long. Length of insect from vertex to tip of cauda, about 2.5 mm., but with much variation.

In general outline the intermediate conforms much more closely to the wingless insect than to the pupa, being plump and of regular outline without having the thorax sharply delineated.

Color characters: In color characters this form resembles the wingless female very closely. In most specimens the rudiments of the wings are of a light green color,

nearly the color of the abdomen, while in some others they are a dusky gray. In specimens that have wings as large as the normal hind wing of the winged form, these wings are transparent like those of the winged. In other color characters this form resembles the wingless female.

COMPARISON WITH USUAL FORMS

Up to and through the pupal stage these insects appear to be identical with the immature stages of the true winged aphides. In fact, the writers are not able to distinguish the pupal molts from which intermediates emerged from those shed by the winged insects.

The adults, however, more closely resemble the wingless individuals than the winged, in general bodily outline. They lose the "corseletta" of the thorax, which latter at the same time becomes less distinctly differentiated from the abdomen, conforming quite closely to the wingless form. The darker color is also lost, the head and thorax being concolorous with the abdomen.

Two indications of the winged character are retained, however. These insects bear rudiments of wings, varying from wings of nearly half size, with indications of some of the veins, to tiny pads which are hardly more than wrinkles of the skin. Also the antennæ of this form bear, on the third segment, sensoria like those of the winged insects, which are absent in the wingless form. These, however, are not normal, in that usually the entire six are not present, the numbers on the two antennæ vary, and the sensoria are not of uniform size, very few being as large as the normal ones.

One other interesting point is that the dorsoventral muscles of the thorax, which are developed in connection with flight, are very much reduced in all specimens and the longitudinal thoracic muscles are reduced in varying degrees, the amount of reduction in both cases coordinating quite closely with the reduction exhibited by the wings. The writers (19) believe these intermediates to be variants between the winged and wingless forms, and of perfectly normal occurrence, illustrating the steps by which the wingless condition has been attained in the Aphididae.

OCCURRENCE

Intermediates were of rather common occurrence, being observed, as stated above, in 16 experiments. In all, 31 individuals were found.

LENGTH OF NYMPHAL LIFE

The nymphal period was of the same length as that of the winged form. In fact it was impossible to distinguish between the two forms in any manner, until the adult condition was attained.

REPRODUCTION

Reproduction was perfectly normal. Both wingless and winged forms were produced, though the percentage of wingless forms was a little greater than by the wingless mothers. Three adults produced 81 young, an average of 27. This is much below the average for the other forms, but only 3 insects were used, and there is nothing to indicate that, normally, this form would not produce at least as many young as the winged mothers. The average daily reproduction was 2.13 for these three individuals, this being somewhat less than that of either of the other forms. Here, again, however, the small number of mothers detracts from the comparative value of the figures.

LONGEVITY

The average length of life for these three insects was 24.3 days, one living 27 days.

COMPARISON OF THE THREE FORMS

NYMPHAL STAGES

All three forms agree in having four immature stages, the first three existing for equal periods, while the last stage is about two days longer in the winged individuals and intermediates than in the wingless ones.

REPRODUCTION

Table II gives a comparison of the reproductive activities of the three forms.

TABLE II.—*Comparison of the reproductive activities of the three summer forms of Aphis pomi*

Form.	Number of insects.	Average per insect.				Average per day.				Maximum per insect.	Maximum per day.
		First period.	Second period.	Third period.	Season.	First period.	Second period.	Third period.	Season.		
Wingless	80	55.4	.....	.....	.....	2.95	.....	.....	.....	.....	.....
Do	113	.....	30.9	.....	.....	.....	1.92	.....	.....	.....	.....
Do	23	.....	.....	12.1	.....	.....	.....	0.83	.....	.....	.....
Do	217	.....	.....	.....	37.5	.....	.....	.....	2.22	133	16+
Winged	29	50.1	.....	.....	.....	2.92	.....	.....	.....	.....	.....
Do	25	.....	25.4	.....	.....	.....	2.04	.....	.....	.....	.....
Do	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	54	.....	.....	.....	39	.....	.....	.....	2.62	120	6
Intermediate	3	.....	.....	.....	24	.....	.....	.....	2.13	27	5

It will be noticed that a comparison of the figures for the entire season gives the winged form a larger average reproduction per insect than the wingless. This is because no winged individuals occurred during the third period when the number of young produced was very low. Eliminating this factor we find that for the first two periods the average for wingless insects was 43+ young, while for the winged aphides it was only 37.7. Unweighted averages have been used here, since it is desired to compare merely the production by the two forms under similar conditions, and the fact that wingless insects were so abundant during the second, or poorer, period would make the use of weighted averages unfair.

PRODUCTION OF SEXES

Both wingless and winged viviparous females may, in addition to producing viviparous females, produce the sexes. However, the wingless individuals are much more commonly sexuparous than the winged insects, since sexes were reared from only three individuals of the latter form.

DIMORPHIC REPRODUCTION

No exact data are available on which to base statements as to the prevalence of dimorphic reproduction—the production of two different forms by one mother. Nevertheless, enough data are at hand to show that it is of very frequent occurrence during the summer and may even be the rule. In several cases wingless, winged, and intermediate mothers produced both wingless and winged offspring. In many cases the first young produced were all wingless, while later progeny were winged; but this was not always true, since the very last young were sometimes wingless.

In a very few cases wingless mothers produced both viviparous and oviparous females, and in one or two instances both males and oviparous females. Again, in a few cases it was possible to determine that one mother produced both oviparous females and males, while in one instance a single viviparous insect produced viviparous females, oviparous females, and males. The production of oviparous females and males by the same mother is probably of quite common occurrence, but the dimorphic reproduction, including both agamic and sexual forms, appears to be rare. In the vast majority of cases one mother will produce only viviparous females or the sexes. It is of interest to note that in most of the cases recorded the agamic young were produced first and the sexes were the last forms produced by the mother.

OVERLAPPING GENERATIONS

Since the writers did not select the first and last young from each mother, they did not obtain exact data on the duration of each generation. However, using the average length of life of the various generations in

conjunction with their observations they can very closely approximate the true conditions.

In the diagram (fig. 2) the solid lines represent actual records. The hatched lines occurring at the beginning of the fifteenth to nineteenth generations are theoretical. They are necessitated by the fact that the earliest progeny was lost in some of these generations and it was necessary to continue with later offspring. The hatched lines at the end of the several generations are deduced by adding the average length of life to the date of last production of young.

It will be noted that theoretically all the generations from and including the seventh should be expected to produce the sexes. It is quite probable that such production occurs in nature, and that the sole reason sexes

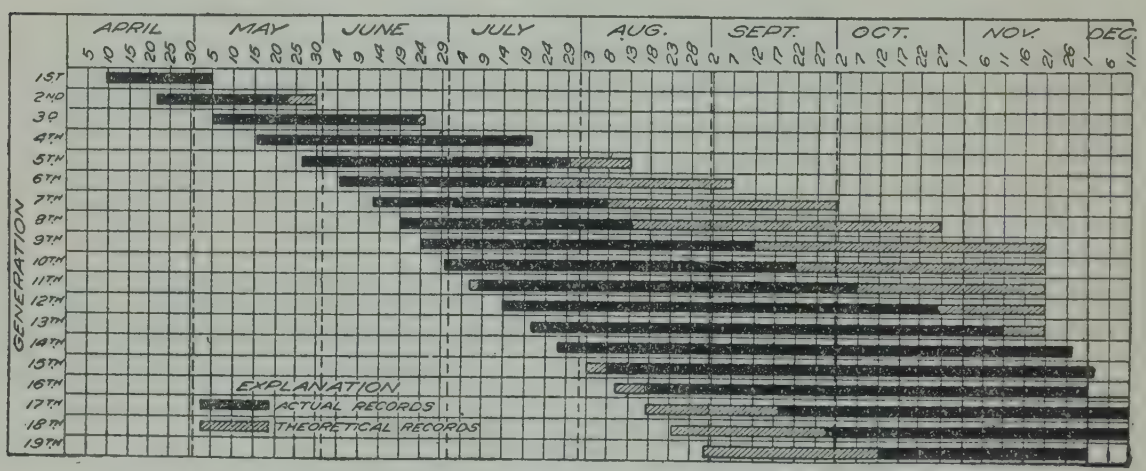

FIG. 2.—Diagram showing the overlapping generations of the green apple aphis.

were not obtained from the seventh, eighth, and ninth generations is that later members of these generations were not reared and bred from.

FEEDING HABITS

As noted previously, the stem mothers fed only on the exposed green of the bursting buds and tiny leaflets, as this was the only food available to them. Later generations preferred the leaf petioles and then the young, newly formed twigs, although some remained on the leaves. In cases where the latter were excessively downy, however, the young stages especially appeared to find some difficulty in living on them. This character of downiness seemed to be particularly unfavorable when occurring on the underside of the leaves. Later, when the twigs commenced to harden, the aphides migrated back to the underside of the leaves, and in the fall, at the time the sexes began to appear, practically no viviparous aphides were found in any other location on the trees. This selection of food occurred only when the numbers were comparatively small. In the case of excessive infestation, twigs, leaf petioles, and the underside of the leaves are attacked simultaneously. Occasionally a single aphis will be found feeding on the upper surface of a leaf, but these cases are so rare as to be almost negligible.

In the writers' experiments the feeding of this species caused very little leafcurl. In the field, however, it often induces considerable curling, and some writers have recorded the injury as being very severe. This injury appears to be produced mainly by the earlier generations. The writers have had under observation some old trees whose water sprouts were heavily infested from the middle of the summer to the close of the season. Very few of the leaves on these suckers showed any curling and these few were only slightly affected, being merely rolled over somewhat. Certainly the curling produced by this species (Pl. LXXV, fig. 1) is never as severe as that caused by *A. malifoliae.*

It is very interesting, in this connection, to note that in the spring we seldom found large, pure colonies of *A. pomi* occurring on the trees. In practically every instance there were some individuals of *A. malifoliae* present. Since a single half-grown stem mother of the latter species can cause very severe curling it seems probable that many of the records of this effect from the feeding of *A. pomi* should properly be referred to the rosy apple aphis.

This species has been reported as attacking and injuring young fruit in some cases, and in severe infestations young aphides are often found clustered on the apples. A few experiments were performed along these lines, but the insects could not be induced to feed on the fruit in any instance, even when all foliage was removed from the twig. It seems very probable, therefore, that such feeding is rather rare.

The quality of the food has a very marked effect upon the size, color, and rapidity of growth of the insect (Table III). When furnished with tender succulent food throughout larval life, the adults are large, plump, and light green in color. On the other hand, if the food is poor in quality, the adults will be smaller, dark green, and the bodies will be much wrinkled. The insect will also require a considerably longer period to attain maturity on poor food.

TABLE III.—*Effect of food on rapidity of development and reproduction of Aphis pomi*

Poor food, insects small.				Good food, insects large.			
Experiment No.	Date born.	Nymphal period.	Number of young produced.	Experiment No.	Date born.	Nymphal period.	Number of young produced.
		Days.				*Days.*	
1559.....	Aug. 5	10	15	1617.....	Aug. 13	7	.....
1643.....	Aug. 14	10 to 12	8	1687.....	Aug. 19	7 to 8	28
1645.....	...do.....	10 to 12	14	1839.....	Sept. 1	7 to 8	20
1488.....	July 28	11 to 13	44	1754.....	Aug. 21	7 to 8	25
1660.....	Aug. 17	12+	.....	1807.....	Aug. 27	7 to 8	25
1852.....	Sept. 10	12 to 14	.....	1856.....	Sept. 2	8 to 9	23
Average.....		11.5	10.25	.....		7.7	24.2

It will be noted that in general the smaller forms occurred earlier in the year than the large ones, at a time when the average length of the nymphal period was particularly short; also that, while the percentage of young produced by the larger insects is below the seasonal average, it is, on the whole, higher than the average of the period in which the insects occurred.

It is very difficult to judge exactly the condition of the food supplied to the insects. The size of the leaves furnishes no criterion as to the amount of food available. The aphides do as well on young, newly opening leaves as on larger ones. In fact the largest, plumpest aphides reared were fed on such foliage, while the poorest conditioned insects were raised on old, dark leaves, whose general condition can perhaps best be described as "hard."

Some of the dormant trees used in the spring continued to live throughout the season. These furnished very satisfactory food at first. They put out slender twigs which never hardened and the leaves of which never fully unfolded. During the latter part of the summer, while the foliage continued perfectly green and appeared to be very succulent growth practically ceased. Aphides confined on these plants grew slowly and never attained the size or plump condition of the average adult.

SEXES

OVIPAROUS FEMALE (PL. LXVII, FIG. 4)

DESCRIPTION

FIRST INSTAR.—Morphological characters: Antennæ as follows: I, 0.025 mm.; II, 0.032 mm.; III, 0.096 to 0.128 mm.; IV, base 0.042 to 0.056 mm., unguis 0.088 to 0.12 mm.; segments I and II with stout spinelike hairs, III and IV imbricated and bearing similar spines; segment III with a distal sensorium, and IV with the usual sensory group. Compound eye with about 14 facets. Labium about as long as the antennæ. Legs hairy, hind tibiæ about 0.209 mm. long.

Color characters: Very variable, usually an olive green, with dusky appendages.

SECOND INSTAR.—Morphological characters: Antennæ as follows: I, 0.028 to 0.042 mm.; II, 0.028 to 0.042 mm.; III, 0.06 to 0.112 mm.; IV, 0.048 to 0.08 mm.; V, base 0.058 to 0.08 mm., unguis 0.12 to 0. 16 mm.; segment IV with a distal sensorium, and V with the usual sensory group, otherwise quite similar to antennæ of last instar. Compound eyes with about 24 facets. Labium nearly as long as III and IV of the antennæ. Cornicles thick, rounded at the tip. Legs more slender than in the previous instar; length of hind tibiæ, 0.256 to 0.32 mm.

THIRD INSTAR.—Morphological characters: Antennæ as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.16 to 0.176 mm.; IV, 0.109 mm.; V, base 0.08 mm., unguis 0.184 to 0.208 mm.; segments armed similarly to those of the previous instar. Compound eyes with many facets. Cornicles more cylindric than in the previous instars, 0.112 mm. long. Legs slender, hind tibiæ 0.112 mm. long.

Color characters: As in previous instars.

FOURTH INSTAR.—Morphological characters: Antennæ as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.096 to 0.16 mm.; IV, 0.08 to 0.152 mm.; V, 0.096 to 0.144 mm.;

VI, base 0.08 to 0.096 mm., unguis 0.192 to 0.256 mm.; segment V with a distal sensorium, segments III to V imbricated and with a few prominent spines. Compound eyes large and with very many facets. Cornicles cylindric, 0.161 mm. long, imbricated. Legs slender, hind tibiæ 0.537 mm. long. Cauda conical, this and the anal plate densely setose.

Color characters: Approaching those of the adult, the dark green transverse band apparent in some cases, and the black portions more strongly developed than in the previous instar.

FIFTH INSTAR (ADULT).—Morphological characters: Antennæ as follows: I, 0.064 mm.; II, 0.064 mm.; III, 0.176 to 0.192 mm.; IV, 0.112 to 0.16 mm.; V, 0.144 to 0.176 mm.; VI, base 0.096 mm., unguis 0.24 to 0.288 mm.; segments III to VI imbricated and with a few rather prominent spinelike hairs, without sensoria excepting the usual distal one on V, and the sensory group at base of unguis. Vertex very slightly rounded. Compound eyes large, with distinct ocular tubercles; prothoracic tubercle very large and distinct; abdominal tubercles small with the exception of the first cephalic pair and the pair caudad of the cornicles. Cornicles (Pl. LXXIV, fig. 14) subcylindric, tapering distad, imbricated and slightly flanged. Legs slender, and armed with stiff hairs. Hind tibiæ slightly curved, very little, if at all, swollen, and armed with circular sensoria; these vary greatly in number, from a few to about fifteen (Pl. LXXIV, fig. 20). Three or four seem to be more common than the large numbers. They are very irregular in size, and are often very faint. Anal plate rounded, densely setose, and covered with a few long curved hairs on each side. Cauda somewhat elongate, conical setose, and armed with six or seven curved hairs on each side; length, 0.16 mm. Length of insect from vertex to tip of cauda, about 1.8 mm.

Color characters: Vertex and top of head dark brown to black. Thorax yellowish green, slightly pruinose. Anterior portion of the abdomen olive or greenish yellow, that portion just between and anterior to the cornicles dark green, forming quite a distinct band; segments of the abdomen caudad of the cornicles olive or yellowish green; margin of the abdomen with a row of dark markings. Cauda, anal plate, and cornicles black. Tarsi and distal extremities of tibiæ, femora, and antennæ dark brown.

In older specimens which have oviposited, the green band upon the abdomen becomes narrow and in very old specimens the body color often shows dark (dull) red-brown with the transverse band brighter than the remainder of the body. In a few cases the female is not olive or yellowish green as described, but is orange-yellow, of a color very similar to that of the males.

MALE (PL. LXVII, FIG. 2)

DESCRIPTION

FIRST INSTAR.—Morphological characters: Antennæ as follows: I, 0.024 mm.; II, 0.032 mm.; III, 0.096 mm., IV, base 0.056 mm., unguis 0.088 mm.; segments I and II with a few stout bristle-like hairs; segments III and IV imbricated, the third one toward its distal extremity only and both with a few stout hairs; segment III with a distal sensorium, and IV with the usual group at the base of the unguis. Compound eye with 12 to 14 facets, Cornicles short, thick, and rounded at their distal extremities. Labrum about as long as segments III and IV of antenna. Legs thick and very hairy, hind tibiæ 0.19 mm. long.

Color characters: Pale yellowish brown with dusky appendages and with the body often covered with a mealy bloom.

SECOND INSTAR.—Morphological characters: Antennæ as follows: I, 0.024 mm.; II, 0.032 mm.; III, 0.064 mm.; IV, 0.056 mm.; V, base 0.048 mm., unguis 0.096 mm.; segments with the characters of first instar, excepting that the distal sensorium is on segment IV. Compound eyes with about 18 facets. Cornicles short. Legs somewhat similar to those of the previous instar, hind tibiæ 0.192 mm. long.

Color characters: Similar to those of the previous instar. Tarsi, distal extremities of tibiæ, and distal extremities of antennæ black.

THIRD INSTAR.—Morphological characters: Antennæ as follows: I, 0.032 mm.; II, 0.04 mm.; III, 0.112 mm.; IV, 0.08 mm.; V, base 0.064 mm., unguis 0.112 mm. Armament of the antennæ, legs, etc., as in previous instar.

Color characters: As in previous instar.

FOURTH INSTAR.—Morphological characters: Antennæ as follows: I, 0.041 mm.; II, 0.041 mm.; III, 0.08 to 0.144 mm.; IV, 0.056 to 0.128 mm.; V, 0.072 to 0.112 mm.; VI, base 0.064 to 0.08 mm., unguis 0.128 to 0.176 mm.; segments III to VI imbricated and armed with a few stout hairs; segment V with a distal sensorium and VI with the usual group at base of unguis, otherwise the segments are similar to those of previous instar. Compound eyes with very many facets. Cornicles cylindric and imbricated, 0.072 to 0.096 mm. in length. Legs with many prominent spines, tarsi imbricated, tibiæ 0.368 to 0.448 mm. long.

Color characters: General color characters similar to those of third instar. Black marking only on the distal extremities of the antennæ, the distal extremity of the labium, the cornicles, the tarsi, and the distal extremities of the tibiæ.

FIFTH INSTAR (ADULT).—Morphological characters: Antennæ (Pl. LXXIV, fig. 9) as follows: I, 0.045 mm.; II, 0.045 mm.; III, 0.16 to 0.184 mm.; IV, 0.128 to 0.168 mm.; V, 0.112 to 0.144 mm.; VI, base 0.081 mm., unguis 0.184 to 0.232 mm.; segments III to VI strongly imbricated and armed with numerous stout hairs; segment III with 7 to 10 irregularly placed sensoria, the arrangement of these giving the segment a slightly knotty appearance; segment IV with about an equal number of sensoria irregularly arranged; segment V with about 5 sensoria of unequal size and with irregular arrangement; segment VI with the usual group at the base of the unguis. Vertex slightly rounded. Eyes with distinct ocular tubercles; thorax with a very prominent tubercle; abdomen with four lateral tubercles on each side, the pair caudad of the cornicles and the most cephalic pair larger than the others. Cornicles (Pl. LXXIV, fig. 13) cylindric, imbricated, slightly flanged distad, 0.104 to 0.28 mm. in length. Legs slender, hind tibiæ 0.496 to 0.592 mm. long. Cauda conical, not constricted, setose, and armed with long curved hairs. Anal plate somewhat truncate; genital plate rounded, wrinkled, and spiny; claspers irregular, corrugated, covered with minute spines; penis long, curved, fleshy (Pl. LXXIV, fig. 8). Length from vertex to tip of abdomen, about 1.12 mm. Shape of insect elongate and narrow, much more so than any other form.

Color characters: General color greenish brown, occasionally olive, sometimes with an orange tinge. Antennæ, cornicles, cauda, and genital appendages black; crown with a black cap similar to that of the stem mother; tip of the labium smoky to black. Insects sometimes slightly pruinose.

FIRST APPEARANCE OF SEXES

The production of the sexes is governed apparently by two factors, the season (temperature being of prime importance in this factor) and the generation. Of these the first is by far the more important.

The earliest sexes in breeding cages were born on September 2. They were in the eleventh generation, which was also the earliest generation in

which they occurred in the experiments.[1] Yet some viviparous insects of the sixteenth generation had been born as early as August 17, indicating very clearly that the season is of great importance in determining the production of sexual forms.

The evidence supporting the other factor is not quite so direct. The first sexes in the eleventh generation were born on September 2, in the twelfth and thirteenth, on September 5; in the fourteenth, on September 8; in the fifteenth, on September 22; in the sixteenth and seventeenth, on September 24. In all the generations up to and including the fifteenth, viviparous young were born on or before September 3.[2] In the sixteenth generation no young were produced between September 3 and 9, when viviparous young were born. The earliest vivipara in the seventeenth generation were produced on September 19.

The accompanying diagram (fig. 3) gives the curves for percentage of experiments containing sexes, by dates. Each date summarizes the production for seven days, the recorded date being the middle one of the seven. The writers can not give the exact percentage of sexes in each generation, since all of the progeny were not reared. However, of the generations occurring wholly after September 1, the sixteenth contained sexes in 51 per cent of the experiments, the seventeenth in 80 per cent, and the eighteenth in 100 per cent. In the nineteenth generation all the insects produced were oviparous females or males.

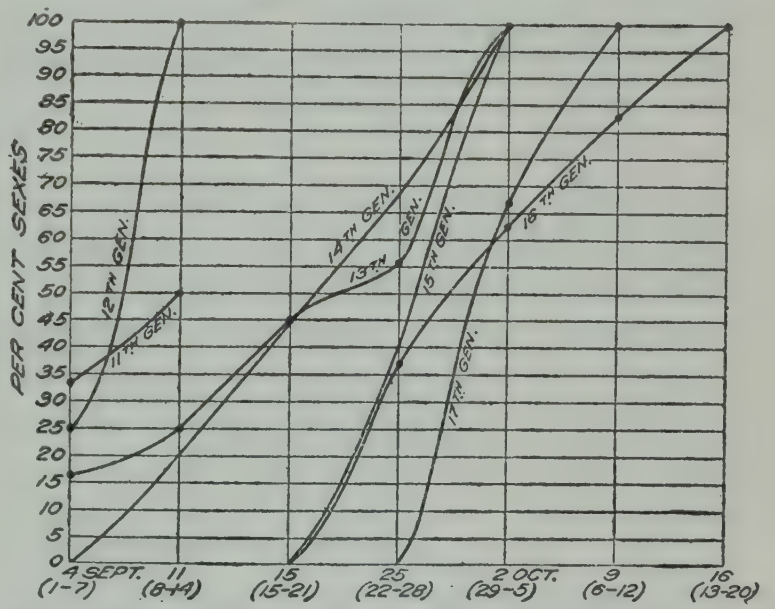

FIG. 3.—Diagram showing curves for percentage of experiments on the green apple aphis in which the sexes appeared.

The most striking points brought out by these figures are that, besides the fact that each generation first occurs at a later period than its predecessor, an additional period is required (to and including the seventeenth generation) for the first appearance of sexes, and that in general the earlier generations are producing sexes in every experiment at a time when later generations are producing them in a very small percentage of experiments. This would indicate that, while seasonal climatic con-

[1] It seems probable that they may occur as early as the eighth generation under some conditions. See page 982.

[2] The insects born in the sixteenth generation before August 17 are not included in this discussion, since they failed to reach maturity, and it was necessary to go back two generations for new material.

ditions are the principal factor in the production of these forms, yet different, perhaps more severe, conditions are needed for each succeeding generation. Also, the generation itself becomes of more and more importance, till in the eighteenth (first produced on September 30) every experiment contains some sexes, while in earlier generations batches of young containing only parthenogenetic females were produced after that date. This latter point is emphasized by the fact that in the nineteenth generation only sexes appeared, while in the earlier generations some viviparous insects were produced as late as were any of the insects in the nineteenth.

It should be stated that the first sexes, in the open, were observed about September 15. These were partially grown. By September 22 adult and nearly full-grown males and females were abundant, indicating that these forms were produced at least as early as the 6th of September.

PERCENTAGE OF MALES TO FEMALES

Notes were not made in every case of the numbers of males and females in an experiment, but the records of 71 experiments in which such figures were kept give an average of 11 per cent of males in a total of 350 insects. This is above the true average, since many experiments contained "many females and no males," and such records have not been included. In only four experiments did the males outnumber the females, and in these experiments the greatest number of sexes raised was six.

LENGTH OF NYMPHAL LIFE

The period covered by the nymphal life of this form was considerably longer than that covered by the same stages of viviparous females, although there were only four nymphal stages, as in those forms. The average period for the immature stages was 20.6 days, the range being from 16 to 36 days. It was impossible to obtain satisfactory data as to the divisions of this period occupied by each stage, as in the majority of the oviparous females the normal rate of growth was considerably deranged by cold spells. Such conditions would greatly retard the development of the insect, with the result that the particular stage in which the insect passed through such temperatures was lengthened in comparison with the other stages. Thus, one experiment might show the first to be the longest stage, while in another the longest stage might be the third. In the case of oviparous females born early in September, the first three stages occupied about the same amount of time as the entire nymphal period of the viviparous females, while the last stage continued for about 6 days. Later in the fall it was impossible to make a comparison. The males require the same amount of time for complete development as do the females and the length of the nymphal period is affected by climatic conditions in exactly the same manner for both sexes.

LONGEVITY

The longest record we have for total life of females is 47 days. At the end of this period the experiment containing two females was set aside and was not examined again for a month. At this time all were dead. The average life for the sexual females is about 25 days. The period varies with climatic conditions, insects born late in the season not living as long as those born in September. The total life period of the male appears to be considerably shorter than that of the female. The longest period observed was 31 days. In this case the male was never transferred from the plant on which it was born, and several females were present. When a male was transferred to a new tree bearing only one or two females, it usually disappeared within a week. In some cases it died, but often it could not be located at all. Toward the end of the season females were still quite abundant, but no males could be found.

The last oviparous females were observed, under natural conditions, on November 27. They were on a tree which still bore five or six green leaves. The next day these leaves fell and no more insects could be found. In the cages living oviparous females were present on January 5, at which time all experiments were closed.

HARDINESS

This species, particularly the oviparous females, can withstand very severe temperatures. On January 5, 1915, observations were made on some experiments in the insectary. These experiments contained both viviparous and oviparous females. At this date all the viviparous and most of the oviparous females were dead. However, on one plant one living insect was found, while a second plant bore six insects which were alive. These latter six were very quiet, showing only the slightest movement when disturbed. The other one, however, was quite active and moved about on the plant. At the time the observations were made (2 p. m.) the temperature was 43° F., and these insects had been subjected to such low temperatures several times, the minimum being −6°.

MATING

The oviparous females may mate within two days, and possibly in less time than that, after reaching maturity. On the other hand, a female may mate for the first time at least eight days after having become adult. The principal factor in determining this point is the facility with which the male finds the female.

Males have lived for considerable periods of time, as much as 10 days, and have spent much of the time on the same leaf with the female, and yet mating apparently did not take place. When males have been placed

beside females, even in contact with them, they have shown no signs of recognition. Sometimes they would remain by the female and commence feeding. Usually they would immediately wander away. Nevertheless, the male appears to be constantly searching for the female. Although it feeds considerably at periods, it is usually engaged in running rapidly about over the plant. The writers have seen such a male pass close to a female, which has produced one or more sterile eggs, several times and not pay the slightest attention to her. Some time later such a female would produce fertile eggs, proving conclusively that he finally found her. It may be that the female is only in condition to mate at certain times and that when not in condition she offers no attraction to the male.

The writers have never witnessed the entire act of copulation. A pair may remain in copula for at least 25 minutes, but whether or not the period is usually much longer than that is uncertain. During mating the female may move about carrying the male with her. She usually remains quiescent, however, with her beak inserted in the leaf or twig on which she rests.

Whether or not plural mating is necessary for fertilization of all the eggs is a point concerning which the writers are uncertain. It is indicated, however, by the fact that in a few cases females have laid fertile eggs and later sterile ones. Certainly plural mating takes place quite frequently. In one case under observation a female mated three times before laying any eggs, the first egg being produced between three and four days after the last mating observed. This is very difficult to explain unless the suggestion that the female mates only when in the proper condition is incorrect, in which case it is possible that the eggs were not fertilized by the first two matings. The writers have never observed females in copula after they have laid fertile eggs, but aphides which have laid sterile eggs frequently mate and produce fertile ones later.

OVIPOSITION

The shortest time observed by the writers to elapse between mating and egg deposition is 2 days. However, in one experiment a female deposited a sterile egg on one day and a fertile one on the next. This would suggest very strongly that oviposition may take place within 24 hours after mating.

In the experiments the number of eggs laid by females ranged from 1 to 6. The normal number appears to be 6, though the average was 4.75. The rate of deposition is very irregular. In one case a female laid 2 in 24 hours and a third in the next 48 hours. In another case a female produced 3 eggs which were laid 6 and 5 days apart. In several cases females which had been observed in copula produced no eggs whatever, although living several days afterwards. On the other hand,

most of the unfertilized females were observed to produce some sterile eggs, frequently laying the entire 6.

During the fall of 1914, eggs were first observed on the trees at Vienna, Va., on September 29. These were newly laid, being still yellow in color.

SUMMARY OF LIFE HISTORY

The life history of *Aphis pomi* may be briefly outlined as follows: The egg is laid upon the tender twigs of the apple, though occasionally it is laid upon the bark of the older twigs. It is light yellow when laid, but later changes to shining black. Development for a few days is very rapid, after which the egg rests for the winter. When the revolution of the embryo is completed in the spring, an increase in temperature will cause the egg to hatch. Before this revolution a high temperature only tends to destroy it. Early in April the egg hatches by a uniform splitting over the insect's head.

The stem mother is wingless and becomes mature in about 10 days. She produces summer forms, both winged and wingless, with the winged ones predominating. There are 9 to 17 generations of the summer forms at Vienna, Va. After the second generation the wingless forms always outnumber the others, but winged forms may occur in every generation. They become rare toward the end of the season. On the other hand, a wingless line may be carried from the stem mother to the egg. A third form, the intermediate, may occur throughout the summer.

The wingless sexes begin to appear about the 1st of September. They occur in all generations, from the eleventh to the nineteenth, inclusive, and probably also in the ninth and tenth.

The summer wingless forms and the oviparous females, which live longer than the males, remain on the trees at Vienna, Va., until the leaves drop, usually about the middle to the last of November.

Mating commences toward the close of September, one male usually serving more than one female. Both sexes feed. The oviparous female may lay infertile eggs if not reached by a male, and these eggs do not become black. The fertile egg develops to the resting stage before the first heavy frosts; otherwise it may be winterkilled and will not hatch to a stem mother the following spring.

GENEALOGICAL DIAGRAM

The accompanying diagram (fig. 4) shows the number of lines possible from one stem mother as indicated by the writers' breeding experiments. A line from each form reproduced in any given generation from known parents was carried until the sexual forms appeared. In some cases the lines indicated either died or were lost. The former are shown by a short transverse line (–) and the latter by (?). It will be seen from the chart that one direct wingless line was obtained from the stem mother

and that a similar wingless line was obtained from the winged offspring of the stem mother. No direct winged line was obtained, and in those where winged individuals were in some numbers intermediates usually occurred also. Each large circle in the chart represents a generation.

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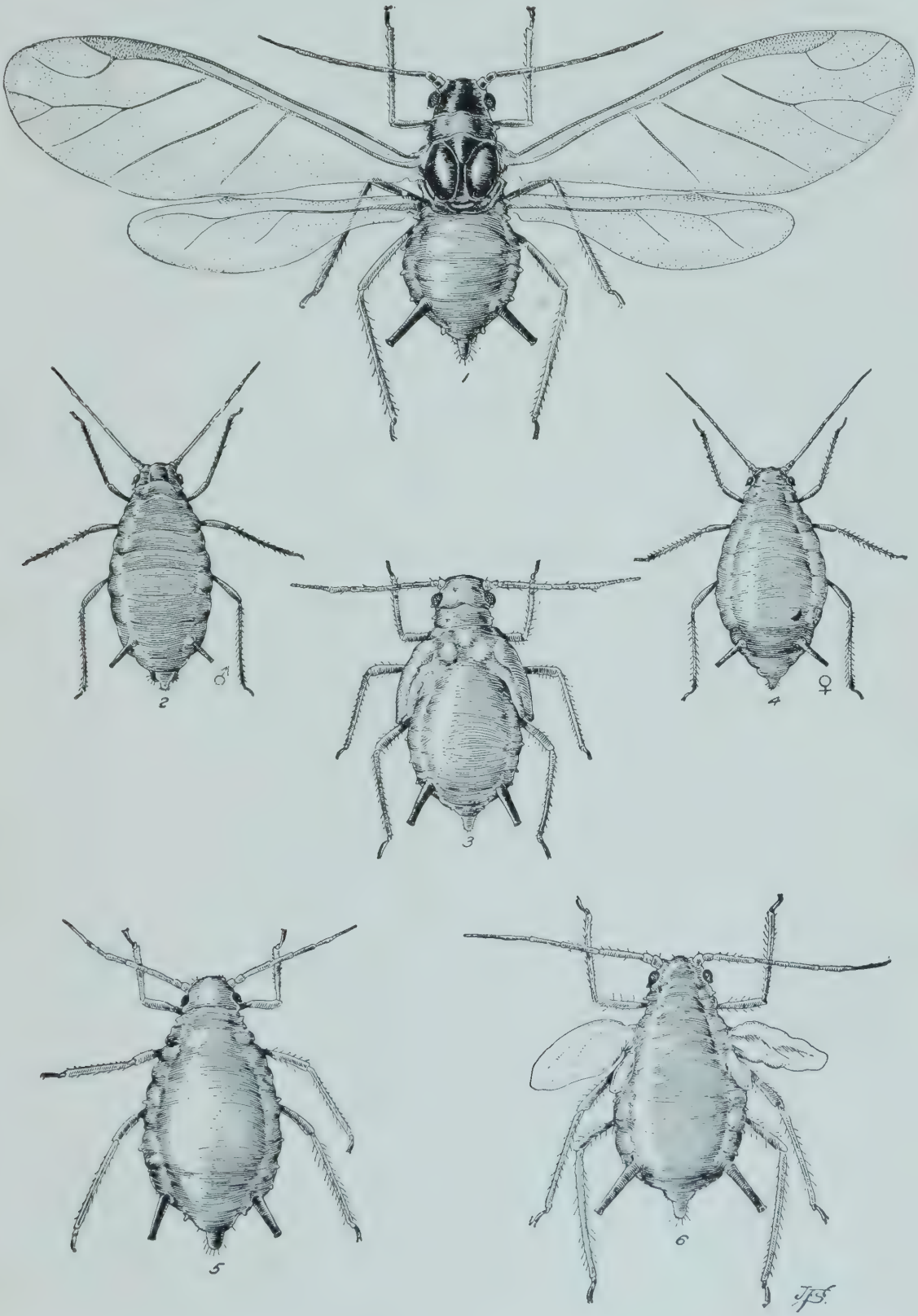
PLATE LXVII

Forms of *Aphis pomi:*

Fig. 1.—Winged viviparous female.
Fig. 2.—Male.
Fig. 3.—Pupa.
Fig. 4.—Oviparous female.
Fig. 5.—Wingless viviparous female.
Fig. 6.—Intermediate.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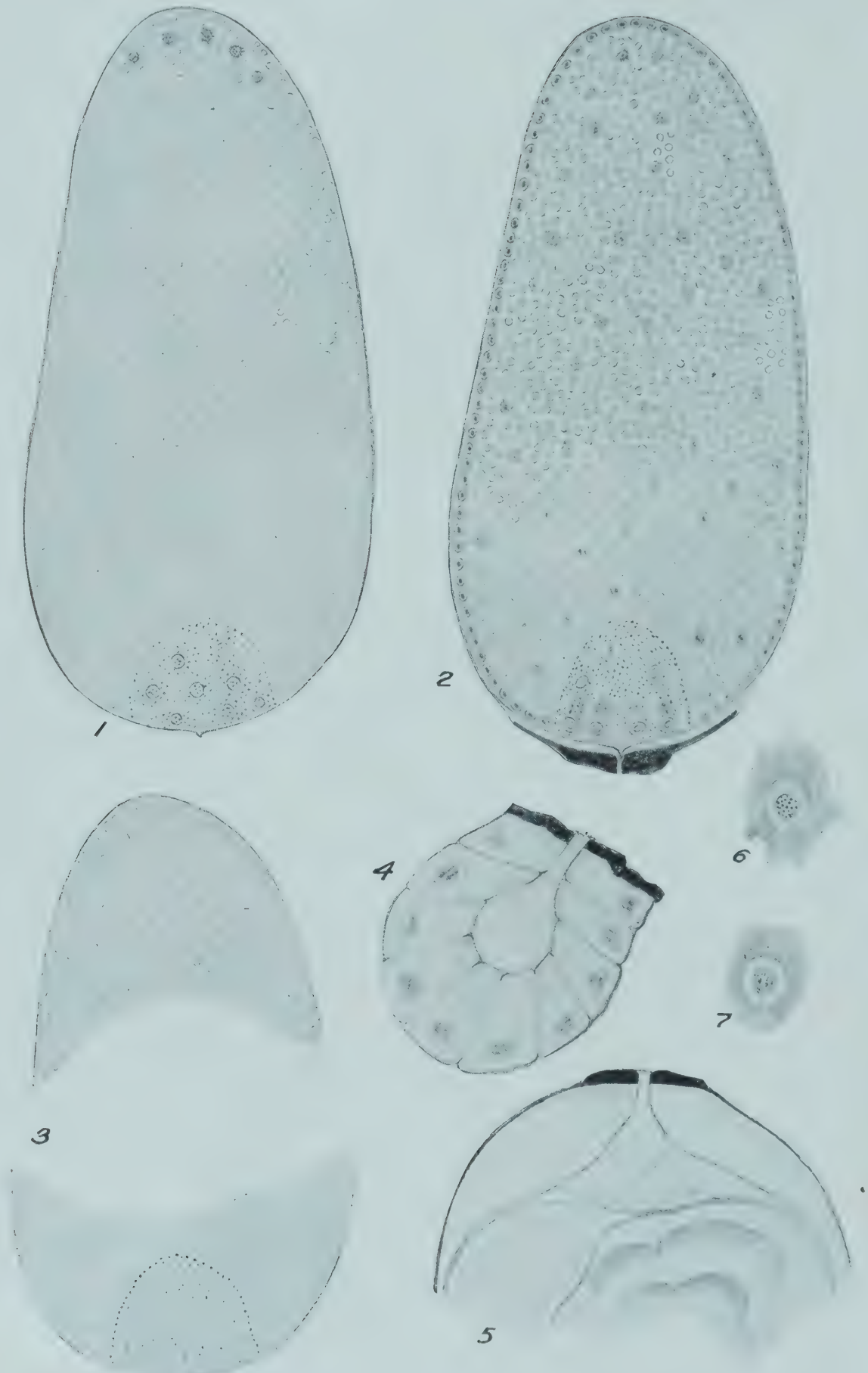
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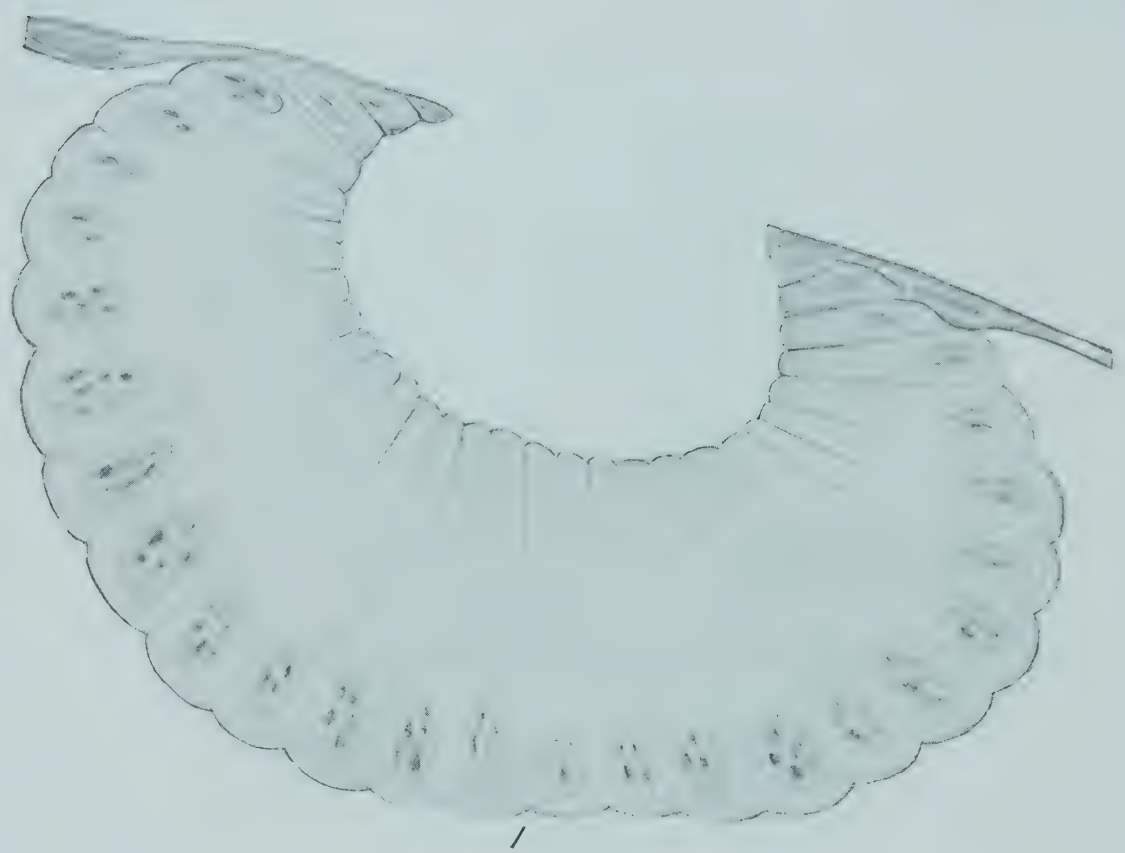

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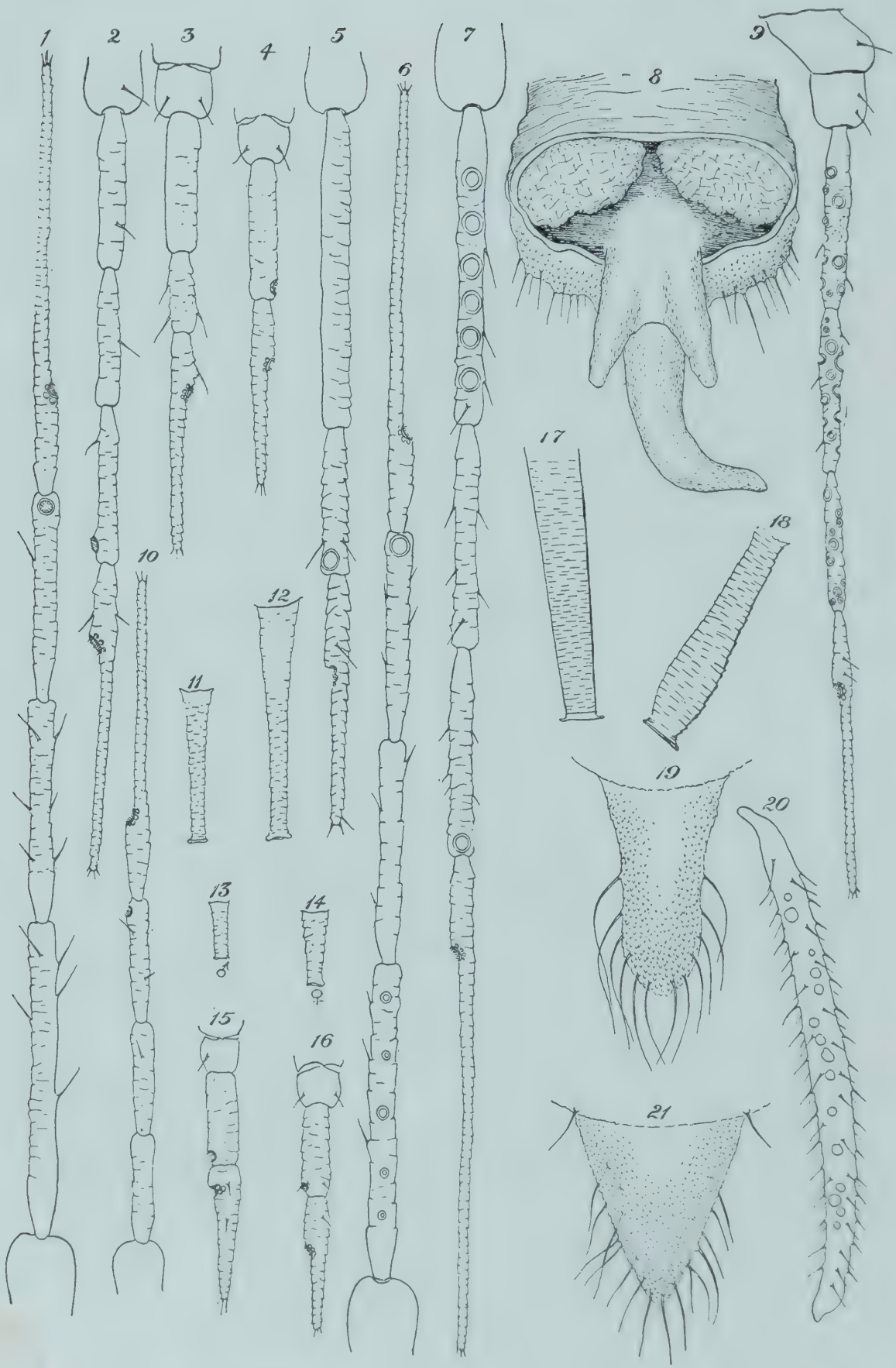
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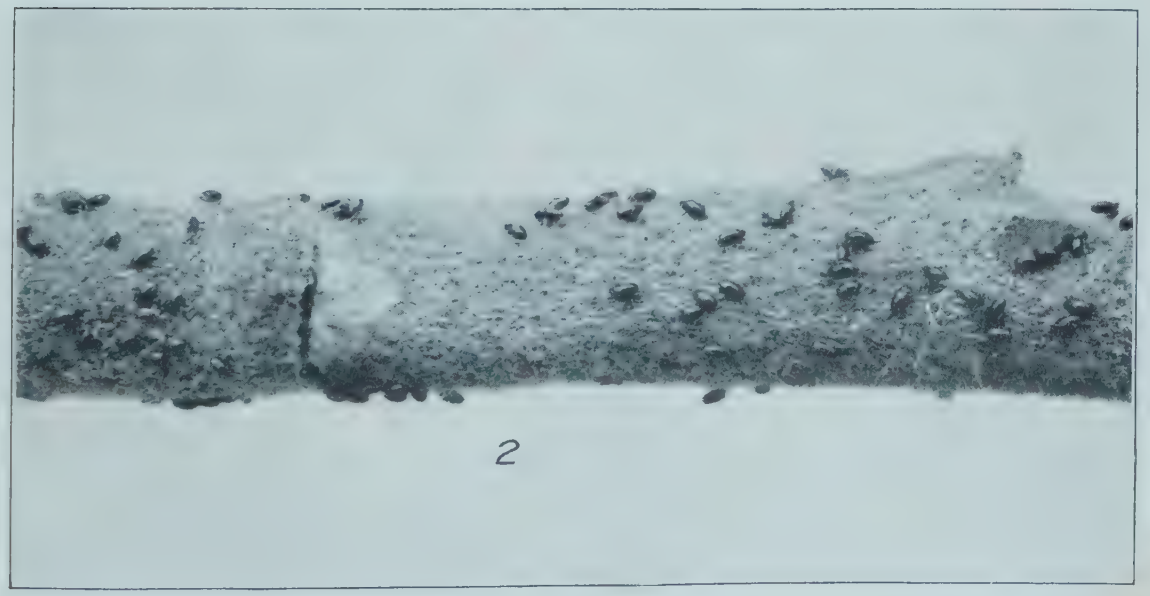

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SOILSTAIN, OR SCURF, OF THE SWEET POTATO[1]

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INTRODUCTION

Soilstain of the sweet potato (*Ipomoea batatas*) is a disease which is little known. The present work is the result of three years' investigations by the writer.

The disease was first described by Halsted (3) in 1890 under the name "scurf." For the last 24 years nothing new has been added to our knowledge of this trouble; subsequent writers have merely quoted Halsted. From the writer's studies (8, 9) it became evident that the disease needed further elucidation. The average grower little suspects that "stain" is a fungus trouble. In fact, the term "soilstain" as applied by the grower indicates his belief that there is something in the soil which stains the roots. He even believes that the plant itself leaves some coloring matter in the soil which stains subsequent crops of this valuable root. Others think that the staining is due to the application of manure to the soil; hence, they term it "manure stain."

ECONOMIC IMPORTANCE OF THE DISEASE

Soilstain is not a disease to be feared in the sense that it may produce a direct rot in the mature roots; nevertheless, it is economically important. Growers whose lands are badly infected assert that stained roots keep better in storage. Others find consolation in saying "there is no such thing as stain, the dark color of the skin being merely a varietal characteristic." The fact remains, however, that many eastern markets discriminate against stained roots. In years of overproduction the New York market refuses stained roots. The western buyers, on the contrary, are lax on this point; otherwise, many growers in the United States would be forced to cease producing sweet potatoes for want of a market.

OCCURRENCE OF SOILSTAIN

Soilstain is prevalent in Delaware on practically all sweet-potato land. It has also been reported from other States where sweet potatoes are grown. The writer has met with it in the sweet-potato districts of Delaware, New Jersey, Maryland, and Virginia.

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[1] The Editorial Committee of the Journal of Agricultural Research kindly forwarded to the writer a copy of Harter's paper on "Sweet-Potato Scurf" before it was published, with the suggestion that reference to that article be made. The writer has covered certain studies on the scurf of the sweet potato in storage and has treated more fully the morphology and physiology of the fungus than has Harter. These studies verify the work of Harter with one exception; in the morphology of the fungus he overlooked the fact that the conidia are catenulate.

[2] The writer is indebted to Dr. Charles Thom, of the Bureau of Chemistry, and Mrs. Flora W. Patterson, of the Bureau of Plant Industry, for having examined specimens of this fungus.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
Vol. V, No. 21
Feb. 21, 1915
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SYMPTOMS OF SOILSTAIN

Soilstain is characterized at first by small, circular, deep-clay-colored spots on the surface of the sweet-potato root. These spots occur singly, but usually there are several in a given area. When very numerous, the spots coalesce, forming a large blotch which sometimes takes the form of a band or may cover the entire root. Soilstain is particularly conspicuous on the white-skinned varieties, such as the Southern Queen. Here the color of the spots is that of a deep-black clay loam. On the darker-skinned varieties the color of the spots does not appear so conspicuous. Soilstain is a disease of the underground parts of the plant. The vine and foliage are never attacked as long as they remain free from the soil. However, when these are covered, the petioles as well as the stems become infected.

EFFECT OF THE DISEASE ON THE HOST

After several months of storage, badly affected roots become a deep brown, which greatly contrasts with noninfected sweet potatoes. Occasionally, badly stained roots seem to be subject to more rapid drying and shrinking. This, however, is not often the rule. Usually soilstain is very prevalent in overheated storage houses. It may be, therefore, that the rapid shrinkage is due to the overheating and not to the effect of the disease itself. More data are necessary to determine these points. Soilstain is not only a disease of the epidermis (Pl. LXXVII, fig. *a*) and as such considerably reduces the market value of mature roots, but it also attacks the very young rootlets, preventing their further development and indirectly reducing the yield. In badly affected fields the writer has estimated a loss of 10 per cent of the crop from rootlet infection.

FACTORS FAVORABLE TO SOILSTAIN DEVELOPMENT

The type of soil seems to be a determining factor in the development of soilstain. Sweet potatoes grown on very light sandy soils, especially those which are hilly, are usually free from the disease. The heavier lands, or those rich in humus, rarely produce a clean crop. The application of manure favors the spread of the fungus and increases the stain. In fact, the manure itself is often a carrier of the disease, since diseased roots of all sorts find their way ultimately to the manure pile. The trouble is also carried directly with the seed stock. These, when planted in the seed bed, will produce 100 per cent of diseased sprouts. Experimental data, as well as extensive observations in seed beds and in the field, all corroborate these statements. Wet weather is favorable to the spread and increase of stain. During wet seasons the disease is more plentiful than in dry seasons.

STORAGE EXPERIMENTS

Growers who do not suspect the fungous nature of soilstain are always at a loss to explain the appearance of the trouble in storage when otherwise healthy roots are brought in. In order to determine definitely the effect of storage on this disease, the following experiments were carried out during two consecutive seasons: At digging time in September, 1913, a diseased field was chosen for that purpose. A large number of roots were selected and placed in hampers in the following ways.

Experiment 1.—Three hampers were filled with roots which to all appearances were free from stain. The object of the experiment was to determine whether apparently clean roots taken from a diseased field will develop stain.

Experiment 2.—Three hampers were filled with roots which showed very slight infection. The spots in these cases varied from 5 to 10 in number and were single and scattered. The object of this experiment was to determine whether the disease would increase in storage and the spots coalesce.

Experiment 3.—Three hampers were filled with roots which were thoroughly stained all over. The object of this experiment was to determine whether badly affected roots would be subject to more rapid drying and shrinkage.

Experiment 4.—Three hampers were filled with well-stained roots. At the bottom was placed a layer of stained roots, followed by a layer of healthy ones, on top of which was another layer of stained roots. Each layer was separated from the other by a narrow strip of paper. The object of this experiment was to determine whether healthy roots in contact with diseased ones will become infected under storage conditions.

Experiment 5.—Three hampers were filled with roots which to all appearances were free from stain and were taken from an adjoining clean field. These were to serve as checks.

All the experimental hampers were placed in a medium-sized potato house which had poor facilities for ventilation. The conditions, therefore, were ideal for the experiment. The hampers were stored for a period of 5½ months.

The results of the above experiments may be summarized as follows: The roots in the first three hampers (experiment 1) remained clean, indicating that clean roots, though coming from an infected field, when stored and protected from contact with stained roots, will remain clean. The roots in the second three hampers (experiment 2) showed an increase in the stain and a coalescence of previously smaller spots. The roots in the third three hampers (experiment 3) seemed to be shrunken most. The roots in the fourth three hampers (experiment 4) indicated that apparently healthy potatoes may become stained when placed directly in contact with diseased roots. The check roots (experiment 5) were all free from stain. The above experiments were repeated in 1914 and 1915. The results obtained did not differ from those referred to above.

CAUSE OF SOILSTAIN, OR SCURF

Halsted (3) was first to attribute the cause of soilstain (scurf) to a fungus, *Monilochaetes infuscans* E. and H. However, Halsted and the later writers have left no record of having experimentally proved the pathogenicity of the fungus. The writer has found no records of its having been grown in pure cultures. Several efforts by the writer to obtain the organism from badly stained roots which were kept in storage at first yielded negative results. Each time the causative fungus was overrun by a varied and rapidly growing flora. Pure cultures of the fungus were finally obtained from plantings of young minute spots. Of 300 such spots, 10 per cent yielded colonies of the causative organism, and these were few in number. The plates were examined every day and it was found that the fungus did not appear until nearly three weeks after culturing. Because of this slow growth, the fungus in previous work was overrun by secondary invaders. The cultural work emphasized the necessity of making a large number of poured plates when working with an apparently difficult organism. The first reference to the fact that this fungus had been grown in culture was made by the writer (8, 9) in 1914 and also recently by Harter (4). Using pure cultures of the fungus, the writer reproduced the disease several times at will.

MORPHOLOGY AND PHYSIOLOGY OF THE FUNGUS

It has been stated that Halsted first named the organism. Although some figures are recorded in Halsted's bulletin (3), yet they are only fragmentary and do not take account of all the various stages of the morphology of the fungus. Halsted's observations of the fungus must have been limited to material on the host. In pure culture the fungus grows very slowly. It is characterized by small darkish round colonies (Pl. LXXVI, fig. 1) varying from one-tenth to one-fifth of an inch in diameter. The growth is floccose at the top, and anastomosed below, having a resemblance to a stroma in the substratum of the medium. The surface growth of a colony resembles that of species of Alternaria and some species of Cladosporium, but differing from these by its restricted slow growth. The surface of the colony of *M. infuscans* has an ashen color, which is also the general appearance of the fruiting. The fungus grows better on vegetable plugs and is at its best on steamed onion and celery stalks. The aerial mycelium is branched, septate, and hyaline when young (Pl. LXXVII, *n*, *w*). With age the mycelial cells turn gray, then black, and become filled with oil globules (Pl. LXXVII, *l*, *r*). The submerged hyphæ are made up of smaller cells which in old cultures swell and take on the appearance of chlamydospores. The conidiophores are distinct from the mycelium (Pl. LXXVII, *a*), and not obsolete, as stated by Stevens (7). From extended observations it was found that conidiophores do not arise in clusters, but are always formed singly

(Pl. LXXVII, *a*, *t*, *u*). They are erect, not branched, and when viewed hastily would be mistaken for setæ of species of Colletotrichum or Vermicularia. Upon a close examination they are found to be made of closely septate dark-celled mycelium, the base of which rests on one or two smaller ones (Pl. LXXVII, *a*). Generally the measurements of the conidiophores vary with the medium used. The host, too, seems to have a determining influence.

In material collected at random from the market or direct from storage the conidiophores appear to be smaller than those taken from artificially infected sweet potatoes. In the latter case, the causative organism seems to possess more vigor, because of moisture under control methods. The average of nearly 500 measurements on various media and on the host shows that the conidiophores vary from 100 to 300μ in length. Great difficulty was experienced in studying the formation of conidia. It is difficult to observe spore formation on storage material. Harter (4) claims that there is but one conidium formed at one time at the tip of the conidiophore. As soon as this conidium breaks off, a new one is formed in its place. The studies of the writer on this point are at variance with those of Harter. The writer finds that the spores are borne in distinct chains. In pure culture the chains break up very readily when moistened and pressed down with a cover glass. The spore chains break immediately when moistened with alcohol, oil, or any other liquid (Pl. LXXVI, fig. 2, *k*, *d*, *b*). The chains of spores do not appear to be held together with any kind of mucilage. However, it was found that when a dry cover glass is carefully placed on the surface of a colony growing in a Petri dish and the latter placed under the microscope, all the stages of spore formation could be studied with much ease. The spores are borne in chains (Pl. LXXVI, fig. 2, *a*, *i*, and LXXVII, *g*, *h*). At first, the protoplasm of the tip of the conidiophore is seen to round up, then a minute bud pushes out (Pl. LXXVII, *c*) and increases in size until a mature spore is developed, which is left standing at the tip of the conidiophore (Pl. LXXVII, *d*). All the succeeding newly formed conidia are formed at the tip of the conidiophore, so that the oldest conidium stands at the farthest end of the chain (Pl. LXXVII, *e*, *f*, *i*). Careful observations of these chains have shown them to be made up of from 10 to 28 conidia. A distinct characteristic of the latter is that they are always guttulate (Pl. LXXVII, *m*), irrespective of the medium used. In some cases the conidia in pure culture appear to be massed in "pockets" around the tip of the conidiophore, as in species of Gloeosporium or Fusarium (Pl. LXXVI, fig. 2, *c*, *e*, *g*, *h*, *j*). However, a close examination will show that this is no definite characteristic of the fungus.

It has been stated that the least disturbance will cause the chains of conidia to break up. In so doing they invariably cluster around the conidiophore, grouping themselves in various ways (Pl. LXXVI, fig. 2,

b, c, d, e, f, g, h). This is observed only when the fruitings of the fungus are seen in a dry state. However, when placed in a drop of water or in any other liquid, the chains of spores break up and scatter over the liquid. The spores (conidia) are 1-celled, hyaline, with a greenish tinge, but never dark or brown. They measure from 15 to 20 by 4 to 6μ. Sometimes a germ tube is produced at the tip of the conidiophore which later bears spores (Pl. LXXVII, fig. *h, j, k, o, p*). Broken-off mycelial cells are also capable of germinating. In this case a germ tube upon which spores are formed is first produced (Pl. LXXVII, fig. *b*). The spores readily germinate in water or in any nutrient medium (Pl. LXXVII, fig. *m, q, s, v, x, y, z*).

An attempt was made to determine whether *M. infuscans* would also cause a rot of the interior of the sweet-potato root. Inoculations made with pure cultures of the fungus in slits made with a sterilized and cooled scalpel showed the organism incapable of causing a rot of the root. It was thought that perhaps the starch or the sugar was detrimental, but the fungus grows well on a starchy medium prepared according to Smith (6, p. 196), although not so well on media rich in sugar. It seems probable that neither the sugar nor the starch restricts the growth of the organism to the epidermis only, but this is done by the enzyms of the host.

TAXONOMY OF THE FUNGUS

The name "*Monilochaetes infuscans*," meaning black bristly Monilia, given by Halsted to the soilstain fungus, remarkably describes the main features of the organism. However, Halsted failed to describe fully either the species or the genus. Saccardo (5) barely mentions the fungus. Neither Engler and Prantl (2) nor Clements (1) nor any other systematic writer on fungi record the genus Monilochaetes. The description given by Stevens (7, p. 597) is incomplete. It was probably taken from naturally infected material, where the chains of conidia are seldom, if ever, noticed, since they are partially broken off with the rubbed epidermis. The conidiophores in such material are often broken down or wanting. From the present studies it seems that the writer is warranted in retaining the names of both the genus and the species of Monilochaetes as used by Halsted. Harter (4), too, decided to retain this genus. The description from a pure culture follows.

Monilochaetes infuscans E. and H.

Spores borne in chains which readily break up; conidia hyaline to greenish, guttulate; conidiophores black, several septate; mycelium first hyaline, then darker with age. The submerged mycelium swells irregularly. Conidiophores, 100 to 300 by 3 to 7μ; conidia, 15 to 20 by 4 to 6μ. The fungus is a very slow grower on artificial media. Parasitic on the sweet-potato root, causing a brown, blotched disease of the epidermis.

SUMMARY

Soilstain, or scurf, is a disease of the epidermis of the sweet-potato root. The disease occurs in every sweet-potato section, East and South, and is probably generally distributed. It is more abundant in the heavier soils, especially where manure is used as a fertilizer.

Soilstain reduces the market value of the mature roots. It reduces the average yield by attacking also the younger rootlets and stunting their development.

Soilstain is a disease of the underground parts of the plant. In storage the disease spreads by contact and is favored by moist, poorly ventilated houses.

The fungus *Monilochaetes infuscans* is difficult to culture, because it is a very slow grower and is readily overrun by associated saprophytes. The conidiophores of *M. infuscans* are distinct from the mycelium, the older growth of which is also dark. The conidia are borne in chains which readily break up when moistened or disturbed.

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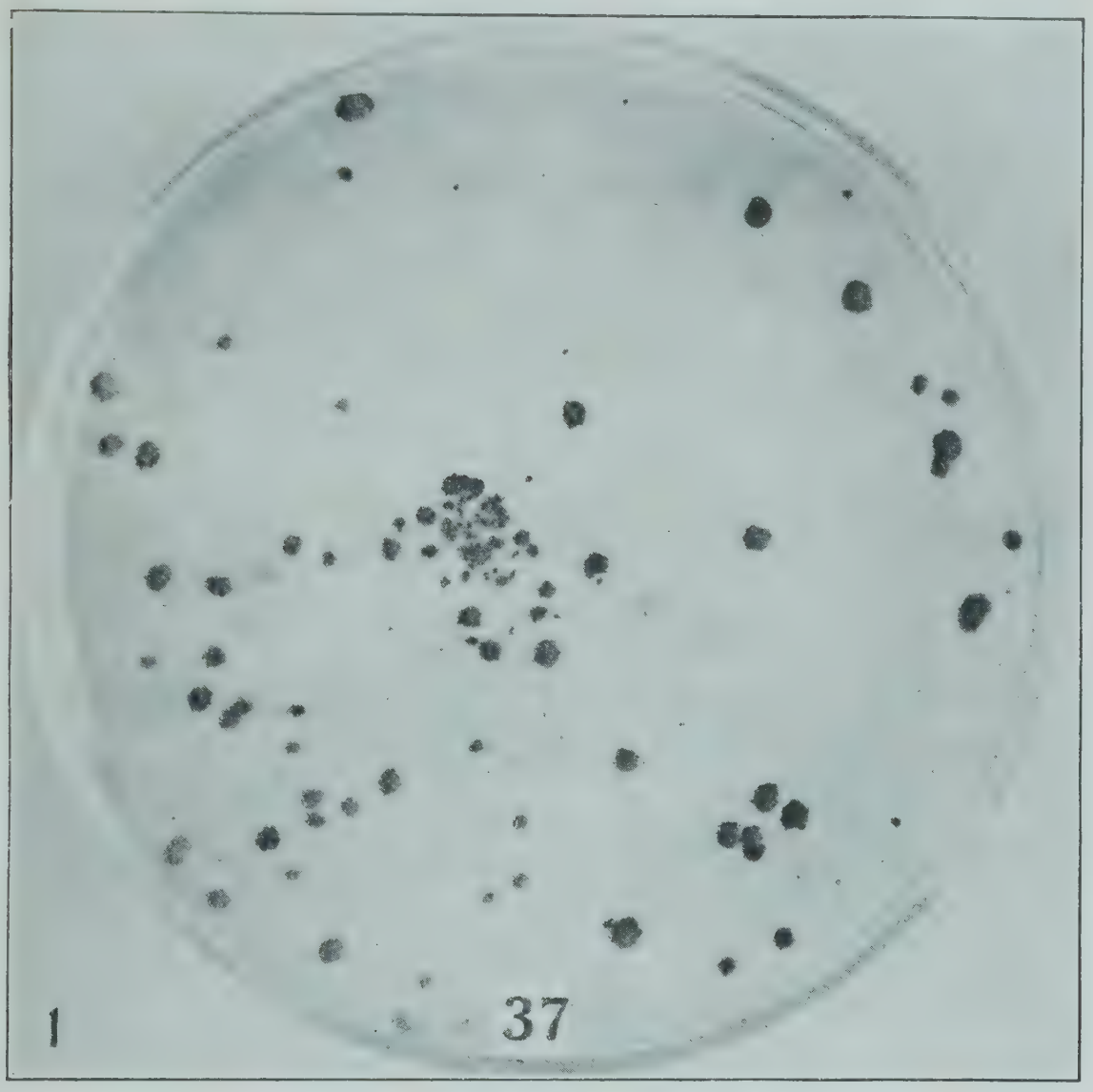
PLATE LXXVI

Fig. 1.—Petri dish containing a pure culture of *Monilochaetes infuscans*.

Fig. 2.—*a*, Part of a conidiophore of *M. infuscans*, showing the unbroken chain of conidia; *b*, *d*, and *k*, various ways of the breaking up of the chains of conidia when disturbed or moistened; *c*, *e*, *f*, *g*, *h*, and *j*, spores collecting in pockets after the chains of conidia have broken up; *i*, bending in of the chain of conidia prior to breaking up into individual spores.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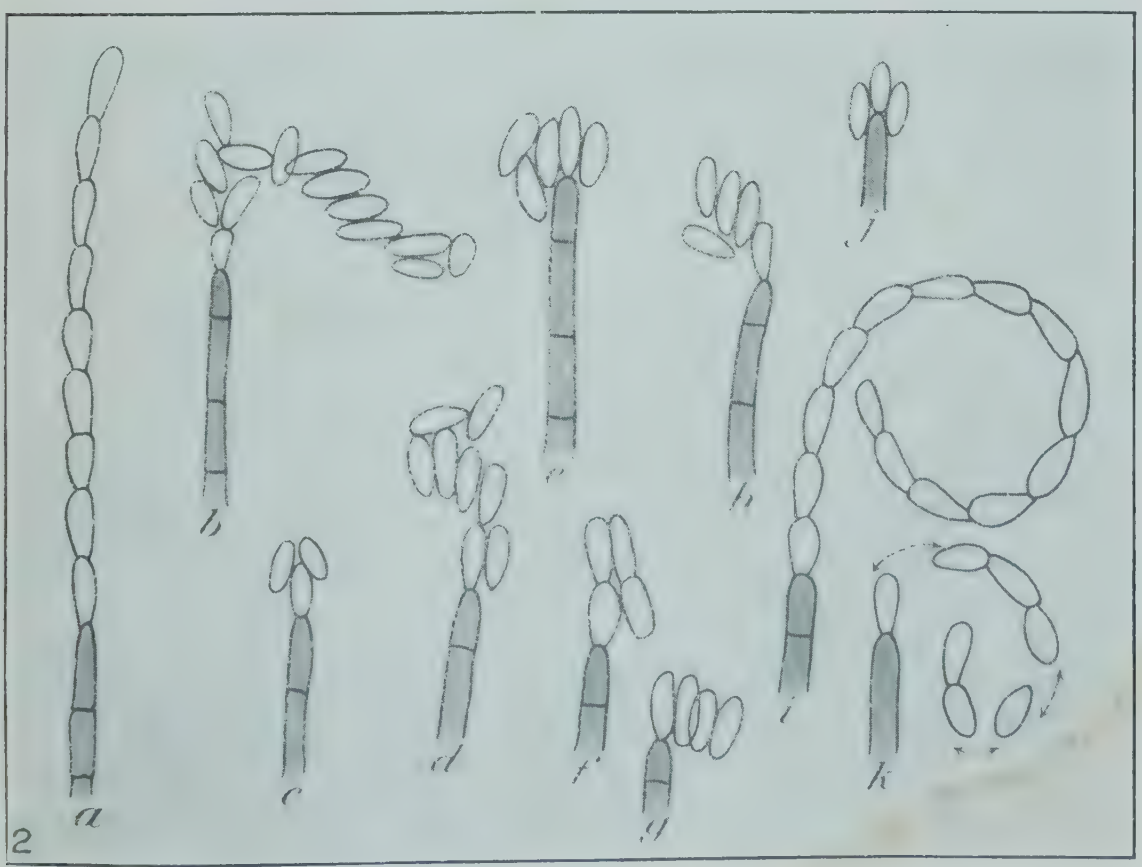
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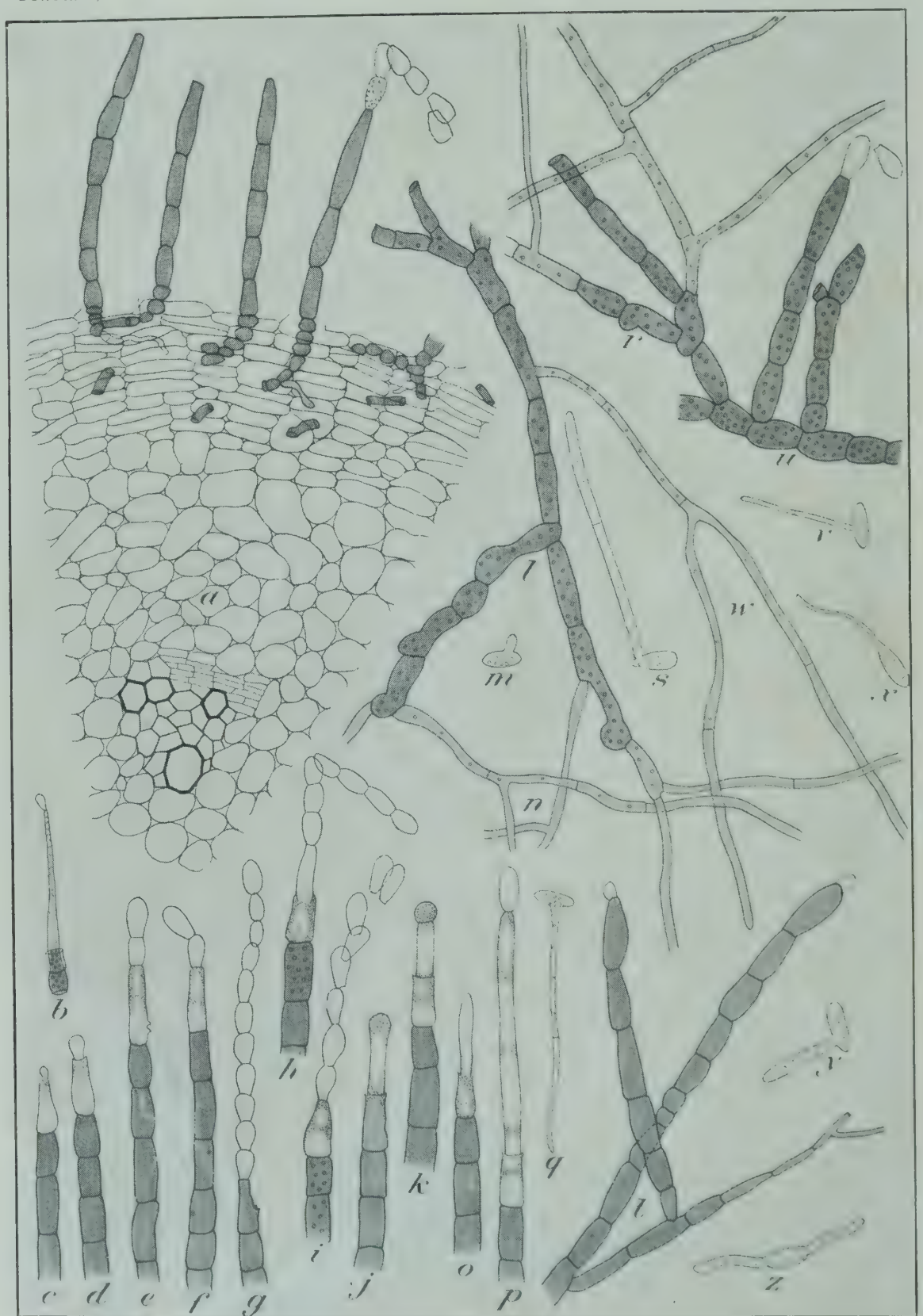
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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., FEBRUARY 28, 1916 NO. 22

AN ASIATIC SPECIES OF GYMNOSPORANGIUM ESTABLISHED IN OREGON [1]

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INTRODUCTION

Early in June, 1914, specimens of a species of Roestelia on Japanese pear leaves were sent to the writer from the office of the Secretary of the Oregon State Board of Horticulture. These had been collected in the yard of a Japanese family at Orient, in the vicinity of Portland, Oreg.

The writer visited the locality on June 11, 1914, and found two Japanese pear trees (*Pyrus sinensis*) the foliage of which was seriously affected with the fungus (Pl. LXXVIII, fig. 1). Since all species of Roestelia, so far as known, are the æcial stages of species of Gymnosporangium, and none are known to be perennial, it was at once recognized that the source of infection must be in the immediate vicinity. A search was made for a possible telial stage, but no positive evidence of the occurrence of such was obtained at that time, on account of the lateness of the season, though several varieties of Juniperus, as well as other members of the Juniperaceae, were found growing in the same yard, all of which were stated by the owners to have been directly imported from Japan several years before. Inquiry revealed that the rust had been present in small amount the previous season.

Careful examination showed that the rust should properly be referred to *Roestelia koreaensis* P. Henn., which was originally described from material collected in Korea (Chosen), but has since been reported as occurring commonly in Japan. An examination of the literature showed that considerable confusion has existed regarding the identity and relationship of certain of the Asiatic species of Gymnosporangium. Two species

This paper is based on studies which were conducted in the laboratory of the Department of Botany and Plant Pathology of the Oregon Agricultural College Experiment Station. It is essentially as read at the summer meeting of the American Phytopathological Society, at Berkeley, Cal., on August 5, 1915, with certain additional information obtained from the examination of material in the herbarium of Dr. J. C. Arthur, to whom grateful acknowledgment is due for this privilege as well as for helpful suggestions.

See *abstracts in* Phytopathology, v. 5, no. 5, p. 293, 1915, and Science, n. s., v. 42, no. 1086, p. 582, 1915.

have been especially confused, and on account of their interest in North America they will be discussed together in this paper. In order to make the situation clear, a review of the literature of these rusts with reference to their occurrence in Japan as well as in the United States will be given.

INVESTIGATIONS IN JAPAN

From 1897 to 1899 Shirai (7)[1] conducted infection experiments in which he claimed to show that *Roestelia koreaensis* was genetically connected with *Gymnosporangium japonicum* Sydow. He succeeded, in several different experiments, in obtaining the development of typical æcia of *R. koreaensis* on the leaves of *Pyrus sinensis* by exposing them to infection from germinating telia on *Juniperus chinensis*. Shirai stated, however, that in Japan the telia of *G. japonicum* occur not only on the trunks and branches, as the original diagnosis of Sydow states, but also on the leaves of the juniper, and he described and figured both stages (7, pl. 1, fig. 19 and 22).

Ito (4) recently called attention to the fact that Japanese mycologists have for some time considered that the forms which occur on the stem and leaves of *Juniperus chinensis* are not the same species. He also recorded the results of infection experiments in which the teliospores of the stem form were sown on *Pyrus sinensis*, *Amelanchier asiatica*, and *Pourthiaea villosa*, with infection only on the last. The resulting æcia proved to be typical of *Roestelia photiniae* P. Henn. Referring to the leaf form, Ito further stated that he considered it to be *G. Haraeanum* Syd. and that *G. asiaticum* Miyabe is synonymous. Miyabe and Yamada (6) have recently shown by infection experiments that *G. asiaticum*, which occurs on the leaves of *J. chinensis*, has for its æcial stage a species of Roestelia on *Pyrus sinensis*, *Cydonia vulgaris*, and *Cydonia japonica*. Hara (3) has also recently shown by infection experiments that *G. Haraeanum* has for its æcial stage *R. koreaensis* on *Pyrus sinensis*.

From the above it would appear that Shirai had both forms, *Gymnosporangium japonicum* and *G. Haraeanum*, mixed in the material which he used for inoculation and that his successful results on the pear were due to infection by the sporidia of the leaf form, *G. Haraeanum* (*G. asiaticum*), and not of the branch form, *G. japonicum*, as was supposed.

OCCURRENCE IN AMERICA

Clinton (1) reported the occurrence in 1911 of *Gymnosporangium japonicum* on imported plants of *Juniperus chinensis* in Connecticut. He also found the two forms on stems and leaves and followed Shirai in considering them identical. Long (5), after a study of Clinton's material, called attention to the difference between the two forms and described the leaf form as *G. chinense*, considering it distinct from *G.*

[1] Reference is made by number to "Literature cited," p. 1009.

Haraeanum. Clinton (2) later admitted that he confused two species, but believed Long not justified in describing the leaf form as new and considered *G. chinense* Long as synonymous with *G. Haraeanum*.

The branch form, *G. japonicum*, has recently (May 19, 1915) been collected on the campus of the University of Washington, at Seattle, Wash., by Dr. J. W. Hotson, and a specimen of it is in the herbarium of Dr. J. C. Arthur and has been examined by the writer.

OCCURRENCE IN OREGON

In the spring of 1915 (Mar. 29) the writer again visited the locality from which he had previously collected the material of *Roestelia koreaensis*. Within 20 feet of the two Japanese pear trees which had shown the infection the previous season and about midway between them two trees of *Juniperus chinensis* were found which showed abundant infection on the leaves of a telial stage of a species of Gymnosporangium. This was determined as *G. Haraeanum*. At the time the collection was made most of the sori had become swollen into gelatinous masses of characteristic shape (Pl. LXXVIII, fig. 3), though a few were found which had not become expanded (Pl. LXXVIII, fig. 2). No other species of Gymnosporangium was found in the vicinity, and no evidence of a branch form was noted.

A considerable quantity of this material was taken to the laboratory of the Department of Botany and Plant Pathology at the Oregon Agricultural College and used in greenhouse infection experiments. No plants of *Pourthiaea villosa* were available, but four potted plants of *Pyrus sinensis* and one each of *Pyrus communis* and *Cydonia vulgaris* were used in the experiments.

The method used was that of suspending branches of the infected juniper over the trees and covering them with large bell jars. This was done on March 30. These were left over the trees for four days, during which time the jars were removed for a few moments daily and the foliage and the inside of the jars sprayed with water. The plants were left covered longer than was intended, it having been the original plan to leave them covered only two days. At the time they were removed it was noted that evidence of infection was already visible on the foliage of the Japanese pear trees. Three or four days later it was evident that pycnia were developing in great abundance on the foliage of these and a few on the quince. There was evidence of initial infection on the trees of *Pyrus communis*, but no pycnia ever developed; only minute black spots finally resulted.

Fully developed æcia were collected from the infected trees of *Pyrus sinensis* (Pl. LXXIX, fig. 1) and *Cydonia vulgaris* (Pl. LXXIX, fig. 2) on June 3, though they were mature fully three weeks earlier. The resulting æcia were found to agree in all respects with the æcia collected in the field the previous year and with descriptions of *Roestelia koreaensis*.

These results, the writer believes, confirm the opinion regarding genetic relationships expressed by Ito and the culture work of Miyabe and Yamada and of Hara, referred to above. They also serve as additional evidence that Shirai's successful infections were obtained with the leaf form rather than with the branch form.

So far as the writer is aware, this is the first record of the complete establishment of any introduced species of Gymnosporangium in this country, though incomplete evidence of the establishment of the same species in California was brought to his attention through a specimen of *Roestelia koreaensis* found in the Arthur herbarium and collected on *Pyrus sinensis* at Oakland, Cal., July 1, 1913, and communicated by Prof. H. S. Fawcett, of the California Experiment Station. Correspondence with Prof. Fawcett and Prof. W. T. Horne, also of the California Experiment Station, revealed that the specimens came from a nursery conducted by Japanese, and that among other things various oriental evergreens were grown. The pears were said to have been originally imported from France in the dormant condition. The presence of this fungus on the leaves of the pears under the conditions is proof that the telial stage must have occurred on some species of Juniperus in the immediate vicinity, though no observations or collections were made. It is evident from this that the rust was at least temporarily established in California at that time.

TAXONOMIC CONSIDERATION

Based upon the rèsults of the infection experiments discussed above, together with the evidence presented in the literature and such studies as the writer has been able to make with the material available in the Arthur herbarium, the present status of the species under discussion is believed to be as follows:

Gymnosporangium koreaense (P. Henn.), n. comb.

Roestelia koreaensis P. Henn., 1899, *in* Warburg, Monsunia, v. 1, p. 5.
Tremella koreaensis Arth., 1901, *in* Proc. Ind. Acad. Sci., 1900, p. 136.
Gymnosporangium asiaticum Miyabe, 1903, *in* Bot. Mag. [Tokyo], v. 17, no. 192, p. (34). (hyponym)
Gymnosporangium Haraeanum Syd., 1912, *in* Ann. Mycol., v. 10, no. 4, p. 405.
Gymnosporangium chinense Long, 1914, *in* Jour. Agr. Research, v. 1, no. 4, p. 353.

Pycnia and æcia on Pomaceae: *Cydonia vulgaris* Pers., reported from Japan and cultured by Miyabe and Yamada; and from Oregon, cultured on June 3, 1915, by H. S. Jackson. *Cydonia japonica* Pers., reported from Japan and cultured by Miyabe and Yamada. No specimens seen. *Pyrus sinensis*, reported from Korea and Japan. (Part of type of *R. koreaensis*, examined.) Cultured in Japan by Shirai, Miyabe and Yamada, and by Hara. Occurred naturally at Orient, Oreg., on June 11, 1914 (H. S. Jackson), and at Oakland, Cal., on July 1, 1913 (H. S. Fawcett). Cultured at Corvallis, on Oreg., June 3, 1915, by H. S. Jackson.

Telia on Juniperaceae: *Juniperus chinensis*, reported from Japan (part of type of *G. Haraeanum*, examined) and from United States in a nursery at Westville, Conn., on stock just imported from Japan on March 28, 1911, by G. P. Clinton (type of *G. chinense*, examined), and from Orient, Oreg., on March 29, 1915, by H. S. Jackson.

Gymnosporangium asiaticum Miyabe is included here on the authority of Ito (4). Regarding *G. chinense*, the writer, after comparing portions of the original collection of this with a specimen of the type collection of *G. Haraeanum*, is inclined to agree with Clinton (2) that they should not be separated. Long (5) gives us the most important basis for separating *G. chinense* from *G. Haraeanum*, the presence of a single apical pore in the upper cells of the former species, found rarely in the thick-walled form, but more commonly in the thin-walled form. He states that in the latter there are two pores in the upper cells always occurring near the septum. A careful examination of a portion of the original collection of *G. chinense* in the Arthur herbarium shows that apical pores occur rarely, even in the thin-walled form, and in every case observed there was a second pore near the septum. The same condition was observed in the type material of *G. Haraeanum*, though rarely. The collection of the writer, made in Oregon, also shows the same condition, but with the apical pores more abundant in the thick-walled form. In all of the collections examined spores were occasionally found in which one of the pores in the upper cell occurred at or near the septum and the other at a point from one-third to one-half the distance from base to apex. The other differences mentioned by Long are largely, the writer believes, due to variation and are not sufficient to justify separation.

Gymnosporangium photiniae (P. Henn.) Kern, 1911, *in* Bul. N. Y. Bot. Gard., v. 7, no. 26, p. 443.

Roestelia photiniae P. Henn., 1894, *in* Hedwigia, Bd. 33, Heft 4, p. 231.
Gymnosporangium japonicum Syd., 1899, *in* Hedwigia, Beibl., Bd. 38, No. 3, p. (141).

Pycnia and æcia on Pomaceae: *Pourthiaea villosa* reported from Japan, cultured successfully by Ito.

Telia on Juniperaceae: *Juniperus chinensis*, reported from Japan and from United States in a nursery at Westville, Conn., on stock just imported from Japan, March 28, 1911, by G. P. Clinton, and at Seattle, Wash., May 19, 1915, by J. W. Hotson.

ECONOMIC IMPORTANCE

Little is known concerning the economic status of the species under discussion. It may be said, however, that any fungus introduced from a foreign land is an unknown quantity and should be treated with suspicion until its status has been established. Several of the American species of Gymnosporangium are already of considerable economic importance, notably *G. juniperi-virginianae* Schw. in the eastern United States and *G. Blasdaleanum* (D. and H.) Kern in the Pacific States.

Gymnosporangium koreaense has been shown to have its æcial stage on the cultivated quince and the Japanese pear. While attempts to infect *Pyrus communis* were unsuccessful, it should be pointed out that only a single attempt was made and it is reasonable to expect that certain varieties of pears, particularly those derived directly or by hybridi-

zation from the oriental species, would be susceptible to infection. It is not known whether this species is capable of infecting the apple. No records of its occurrence on that host have come to our attention.

While the only telial host known for either species is the Oriental juniper, it should be noted that this species is a very variable form, of which many varieties are recognized, and is closely related to several American species of the Sabina group. It is not at all impossible that either of the rusts under discussion might find a congenial host among some of the American species of Juniperus and become firmly established in this way.

The infection experiments of the writer with *Gymnosporangium koreaense* have shown that it develops very vigorously on the quince. Since the species of Gymnosporangium which are known to infect the quince do not usually develop so vigorously on that host as on others, the vigorous growth of this species on the quince may be an indication that *G. koreaense* is rather cosmopolitan in its habits and in a new habitat finally may prove capable of infecting a wide range of pomaceous hosts.

Several of the forms of *Juniperus chinensis* are commonly planted for ornament in various parts of the country, and practically all of these are imported directly from Japan. Both *Gymnosporangium photiniae* and *G. koreaense* are apparently common in Japan and, as shown by the American records, are liable to be frequently introduced on the telial host. If infected trees should be planted in the immediate vicinity of pomaceous hosts capable of harboring the æcial stage, it is possible for either species to become established, as has occurred in Oregon. In the case of the outbreak of *G. koreaense* in the nursery at Oakland, Cal., it is probable that the junipers which were the source of infection for the rust on the pears have been sold and distributed, and the rust may already be established in one or more localities that have not yet come to the attention of plant pathologists.

In the case of *Gymnosporangium photiniae* it is uncertain whether the telial stage is perennial or biennial. Clinton (1) records that an infected tree planted in the greenhouse developed after two years a new sorus in a different part of the stem than the point of original infection. It is known that several other related species which cause fusiform enlargements of the stem are perennial and take more than one season for the development of the telia after infection. As in all species of Gymnosporangium, the infection of the telial host occurs in the summer, and the mature sori do not develop till the following spring or, in some species, until the second spring after infection. *G. koreaense*, so far as known, is an annual form, requiring a new infection of the telial host each year.

In the case of either species it would be difficult to detect the presence of infection during the summer or dormant season, making inspection at the port of entry difficult. To be certain that infected junipers were

not planted, it would be necessary to hold all imported plants in quarantine until the following spring at least, in order to detect the presence of *G. koreaense* and until the second spring for the detection of *G. photiniae*. All trees found diseased should be destroyed, and in case the rust becomes established in any locality it would be advisable to remove the telial host.

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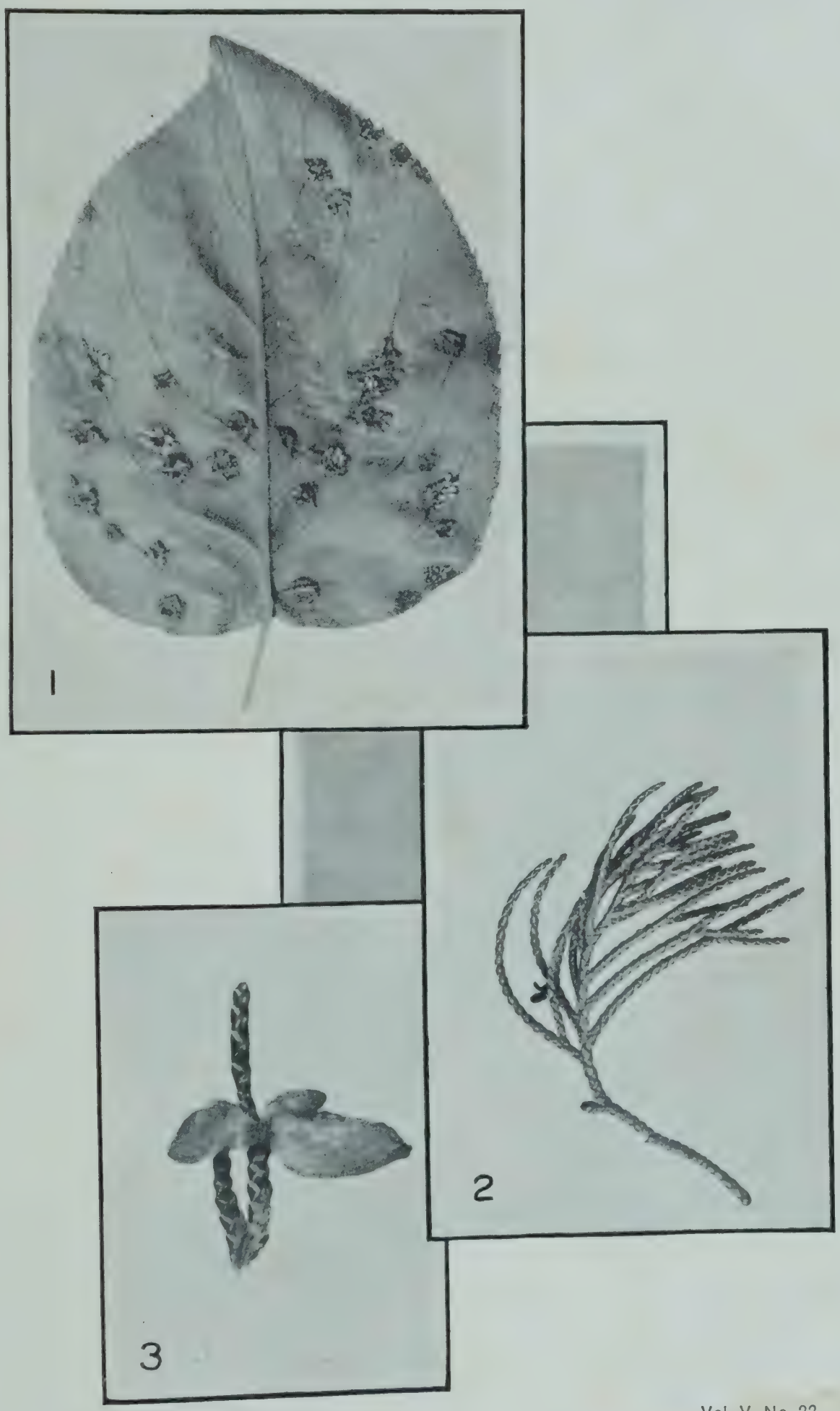
PLATE LXXVIII

Fig. 1.—Æcial stage of *Gymnosporangium koreaense* on under surface of leaf of *Pyrus sinensis*. Field collection at Orient, Oreg. Natural size.

Fig. 2.—Telial stage of *G. koreaense* on young twigs of *Juniperus chinensis*. Sori not distended. Field collection at Orient, Oreg. Natural size.

Fig. 3.—Same as figure 2, with sori distended. ×2.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RELATION OF STOMATAL MOVEMENT TO INFECTION BY CERCOSPORA BETICOLA [1]

By VENUS W. POOL, *Assistant Pathologist*, and M. B. MCKAY, *Scientific Assistant*, *Cotton and Truck Disease Investigations, Bureau of Plant Industry*

INTRODUCTION

Leafspot infection of the sugar beet (*Beta vulgaris* L.) caused by *Cercospora beticola* Sacc. has been found to be closely related to if not directly controlled by stomatal movement in so far as the host is concerned. Penetration of the leaf by this parasite is effected, so far as known at present, only through open stomata. Consequently the factors favorable to stomatal pore opening become of fundamental importance in the occurrence of the disease.

The factors considered in this paper as most important in influencing stomatal movement are leaf maturity and certain environmental conditions. The term "leaf maturity" as employed in this paper is used to designate the condition of those leaves which have reached a maximum degree of physiological efficiency per unit area. Neither the size of the leaf nor its relative age in days can be taken as a reliable index to its degree of maturity. Under certain conditions young heart leaves of the sugar beet may be stimulated into physiological maturity before they have arrived at the average adult size, and such leaves will always remain small, while leaves which have attained average adult dimensions may still be physiologically immature. The varying degrees of leaf maturity have been found to be accurately indicated by the relative size and number of stomata per square millimeter of leaf surface, and these morphological factors have been observed to remain constant for a given maturity, even though the leaf size and position might indicate another stage of development. The stomata on leaves determined as mature by this method exhibited the greatest movement and responded most readily to changes in the environment. Light may be considered the essentially fundamental external factor affecting stomatal movement, although its influence may be greatly modified by different temperatures and relative humidities, the two factors that will be considered in detail in this paper.

In addition to stomatal movement, infection is also influenced by the rapidity of growth of the conidial germ tube and the maturity of the leaves. Detailed field observations have shown that heart and extremely

[1] This study has been carried on in connection with a detailed investigation of the sugar-beet leafspot conducted by the United States Department of Agriculture in cooperation with a beet-sugar company at Rocky Ford, Colo., during 1912 and 1913. A continuation of the entire problem was made possible during the season of 1914 at Madison, Wis., through the kindness of Dr. L. R. Jones, of the University of Wisconsin.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 22
Feb. 28, 1916
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young leaves are not susceptible to infection, and that young mature leaves are only slightiy so, while mature leaves show the greatest susceptibility. It has also been found that old leaves past their maximum development have for the most part lost their susceptibility, for they seldom show an increase in the number of leaf spots present. Thus the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, as they both occur on the leaves of the same degree of maturity.

With the varied host and environmental factors favorable, as might be indicated by the stomata on mature leaves remaining open for a period of from five to eight day hours and with vigorous viable conidia of the fungus present, infection would be practically assured.

FACTORS INFLUENCING STOMATAL MOVEMENT

LEAF MATURITY

A study of the stomata on leaves of different maturities has indicated certain specific characters that might be used to determine the comparative development of different leaves. The number of stomata per square millimeter of leaf surface and the stomatal pore lengths have been found to give a good indication of leaf maturity as determined by the size, condition, and position of a leaf on a normal plant. By using the stomatal numbers and pore lengths as a means of measurement, the degree of maturity of any leaf on a heavily infected or otherwise abnormal plant may be determined, regardless of the degree of development indicated by its size and position. This becomes of especial value in the study of the leaves on a plant heavily infected by *Cercospora beticola*, for the young leaves may be mature, though their size and position would indicate immaturity.

Lloyd's[1] (7) method[2] for observing stomata in situ has been used throughout the study in determining the stomatal numbers and pore openings. Microscopic examinations were made near the middle of the blade of leaves which were taken directly from the plants to the stage of the microscope. Readings were continued not longer than two minutes, the stomata remaining unchanged during that time.

On a normally developed sugar-beet plant, pronounced differences are usually found to exist between the central, or heart, leaves, those occupying a midway position on the plant (here designated as mature leaves) and those occurring at the extreme outer portions of the leaf growth (old leaves). On leaves growing in such relative positions read-

[1] Reference is made by number to "Literature cited," p. 1038.

[2] Lloyd's stomatoscope (shown in Pl. LXXX, fig. 1), which was devised later, was kindly lent by the inventor for the studies which were made in Colorado in 1913. Two characters of this instrument, which make it exceedingly valuable for leaf study, are the long stage and the modified condenser, which serves also as a cooling chamber. The instrument also has a basal screw for tripod attachment. In a letter to the authors he has suggested (1) that the objective should be corrected for use without a cover glass, (2) that the focus of the condenser should be capable of being placed 5 mm. above the stage level for proper use in the case of thick leaves, and (3) that smoked glasses should be provided to shield the eyes.

ings were made of the stomatal numbers and pore lengths, together with the leaf size. These readings were taken during the same period and under comparable environmental conditions and the results are given in Tables I, II, and III, each leaf having been given the same number in all the tables.

STOMATAL NUMBERS

It is shown in the general averages of Table I that the number of stomata per square millimeter of heart-leaf surface (289.8, upper surface; 353.5, lower surface) is more than 2½ times that on mature leaves (100.7, upper; 130.6, lower), as would be expected. There are in turn more on the mature than on the old leaves (80.1 and 105), while cotyledons have the fewest of all (54.7 and 73.2). The plants studied were grown in the field at Madison, Wis., under favorable conditions, and at the time the readings were made they appeared normal in every way. The older plants were about 7 weeks old, and those from which the cotyledons were studied were 3 weeks old. The cotyledons were green and turgid, comparing in maturity and activity probably with those leaves termed "mature." It may also be noted in the averages that more stomata were present on the lower surface of the leaves than on the upper and that the ratio between the two remained uniform.

TABLE I.—*Average number of stomata on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings*[1] *taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
2	.....	.....	92.9 (3)	141.1 (2)	.....	.....	69.7 (4)	54.7 (3)
3	*240.7*[2] (2)	*282.2* (3)	94.6 (4)	124.5 (4)	*53.1* (4)	*78.0* (4)	66.4 (4)	92.9 (5)
4	293.8 (4)	325.0 (3)	94.6 (7)	126.1 (5)	59.7 (6)	102.9 (4)	53.1 (4)	78.0 (4)
5	275.5 (3)	391.7 (3)	104.5 (3)	132.8 (2)	71.3 (3)	86.3 (5)	59.7 (3)	59.7 (3)
6	298.8 (3)	373.5 (2)	*124.5* (5)	129.4 (5)	74.7 (2)	99.6 (3)	*76.3* (3)	.....
7	*353.5* (3)	370.1 (3)	92.9 (3)	*99.6* (3)	94.6 (4)	116.2 (3)	66.4 (4)	109.5 (3)
8	298.8 (1)	381.8 (2)	104.5 (3)	126.1 (3)	83.0 (2)	104.5 (3)	74.7 (2)	107.9 (4)
9	315.4 (1)	381.8 (1)	104.5 (3)	141.1 (2)	89.6 (4)	104.5 (3)	*33.2* (3)	*38.1* (3)
10	307.1 (2)	348.6 (2)	99.6 (3)	121.1 (3)	92.9 (3)	126.1 (3)	49.8 (3)	76.3 (3)
11	320.3 (3)	370.1 (3)	109.5 (3)	137.7 (3)	*99.6* (3)	*132.8* (3)	49.8 (3)	91.3 (4)
12	253.9 (3)	308.7 (3)	104.5 (3)	*154.3* (3)	83.0 (1)	99.6 (1)	38.1 (3)	43.1 (3)
13	.....	.....	.....	.....	.....	.....	49.8 (4)	56.4 (5)
14	303.7 (3)	*398.4* (1)	102.9 (4)	127.8 (4)	.....	.....	58.1 (2)	*126.1* (3)
15	.....	.....	99.6 (2)	.....	.....	.....	49.8 (4)	38.1 (3)
16	.....	.....	99.6 (2)	116.2 (1)	.....	.....	.....	.....
17	249.0 (1)	323.7 (2)	*91.3* (2)	149.4 (1)	.....	.....	66.4 (3)	83.0 (1)
19	.....	.....	91.3 (2)	132.8 (2)	.....	.....	41.5	.....
20	.....	.....	.....	.....	.....	.....	33.2 (2)	49.8 (2)
21	257.3 (2)	340.3 (2)	.....	.....	.....	.....	49.8 (1)	66.4 (1)
Average	289.8	353.5	100.7	130.6	80.1	105.0	54.7	73.2

[1] These leaves were used for the readings given in Tables II, III, and V, and each leaf has the same number in all the tables.

[2] Numbers in italics indicate the maximum and minimum variation.

STOMATAL PORE LENGTHS

The stomatal pore lengths of the different types of leaves show variations that are comparable to those observed in stomatal numbers—i. e., a smaller stomatal size must accompany the greater stomatal numbers per unit area. The pore lengths (Table II) of the stomata on the heart leaves (14μ, upper surface; 14μ, lower surface) are on the average about half that of those on the mature leaves (28.5μ, upper, 27.1μ, lower), and in turn the mature leaves show a slightly shorter pore length than those on the old leaves (31.06μ, upper, and 30.5μ, lower) or cotyledons (31.8μ, upper, and 32.1μ, lower), the last two sets being about equal.

TABLE II.—*Average lengths (in microns) of stomatal pores on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings*[1] *taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
1	.....	.....	33.9 (2)	.....	.....	.....	.....	.....
2	.....	.....	33.9 (3)	27.5 (2)	.....	.....	32.1 (3)	36.6 (5)
3	12.2 (6)	10.5 (6)	29.6 (12)	26.2 (6)	33.9 (6)	33.9 (4)	33.0 (8)	28.3 (9)
4	12.7 (8)	14.0 (8)	25.8 (7)	26.7 (3)	30.9 (6)	29.6 (6)	33.9 (6)	32.5 (7)
5	11.8 (5)	13.1 (6)	28.3 (7)	25.4 (4)	29.6 (5)	29.6 (6)	29.6 (6)	39.8 (7)
6	18.6 (5)	15.6 (4)	32.5 (7)	26.2 (5)	31.3 (7)	30.0 (5)	40.2 (6)	.....
7	15.6 (7)	12.7 (5)	29.6 (9)	26.2 (4)	29.6 (3)	29.6 (4)	38.9 (5)	38.9 (8)
8	12.7 (5)	15.2 (5)	27.5 (7)	27.9 (6)	.....	.....	28.3 (7)	29.6 (6)
9	8.8 (7)	8.8 (6)	26.7 (3)	23.7 (6)	.....	.....	36.2 (3)	39.4 (3)
10	10.5 (4)	8.4 (4)	29.6 (6)	27.5 (4)	.....	.....	29.6 (4)	29.6 (5)
11	16.1 (5)	21.2 (4)	29.6 (12)	29.6 (5)	.....	.....	29.6 (5)	29.6 (4)
12	17.7 (5)	16.1 (5)	29.6 (4)	28.8 (6)	.....	.....	29.6 (4)	29.6 (4)
13	.....	.....	.....	.....	.....	.....	29.6 (6)	29.6 (8)
14	16.9 (4)	16.9 (3)	26.7 (6)	25.8 (7)	.....	.....	30.4 (5)	30.0 (6)
15	.....	.....	28.8 (6)	.....	.....	.....	29.6 (6)	27.5 (6)
16	.....	.....	23.7 (6)	29.6 (5)	.....	.....	.....	.....
17	14.8 (5)	14.8 (4)	27.5 (4)	27.5 (7)	.....	.....	29.6 (7)	29.6 (4)
18	.....	.....	27.5 (5)	27.5 (4)	.....	.....	.....	.....
19	.....	.....	25.8 (9)	27.5 (9)	.....	.....	29.6(12)	31.3 (9)
21	.....	.....	25.4 (3)	27.5 (4)	.....	.....	.....	.....
Average..	14.0	14.0	28.5	27.1	31.06	30.5	31.8	32.1

[1] These leaves were used for the readings given in Tables I, III, and V, and each leaf has the same number in all the tables.

It thus appears that a definite relation exists between stomatal pore length and maturity of the leaf, although at times a shorter pore length might indicate the maturity as being somewhat less than would be shown by the number of stomata present. This may be due to the completed growth of the epidermal cells being attained before metabolic activity reaches its maximum, and consequently the stomatal pore length would be less.

SIZE AND MATURITY OF LEAF

The sizes of the leaves from which the stomatal numbers and pore lengths have been taken show a difference that is characteristic of comparatively young plants during the early summer. As these plants increased in size, the oldest leaves would for a period be normally much smaller than the mature leaves, since the old leaves had been formed at a time when the plants were small. This difference in size is shown in Table III, where the mature leaves are much larger (18.3 by 15.1 cm.) than the old leaves (10.9 by 7.2 cm.), which in turn are only slightly larger than the heart leaves (9.9 by 6.6 cm.). Since the plants had not yet attained their maximum size, these heart leaves would, when mature, probably be larger even than the present mature leaves. Finally, however, a point would be reached where the mature leaves formed would not be increasingly larger with advanced age of the plants, at which time the mature and old leaves should be approximately the same size. It thus appears that there are great variations throughout the season in the sizes of the leaves that are developed at different periods or under abnormal conditions, owing to disease, unfavorable soil factors, etc. However, leaf maturity, regardless of leaf size, may be determined by the number of stomata per unit area and their pore lengths.

TABLE III.—*Comparative sizes (in centimeters) of heart, mature, and old leaves and cotyledons of the sugar beet. Readings [1] taken at Madison, Wis., on July 6, 1914*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons	
	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.
1	.....	.....	18	17	.....	.....	.....	.....
2	.....	.....	18	17	.....	.....	.....	.....
3	10	6	11	16	10.5	7	2.5	0.7
4	14	9	21	16	8.5	7	2.3	.7
5	10	16	20	16	10	7.5	3.0	.8
6	10	5	20	16	17	10	2.0	.7
7	10	5	10.5	7.5	11	7	2.5	.7
8	8	6.5	20	16	8	4.5	3.0	.8
9	8	6.5	20	16	10	5.5	3.0	1.0
10	8	6.5	20	16	10	7	2.0	.6
11	10	3.5	20	16	12	8	2.5	.6
12	12.5	7	20	16	12	8	2.5	.8
14	8.5	4.5	20	16	.....	.....	2.4	.6
15	.....	.....	.....	.....	.....	.....	3.5	.8
16	.....	.....	18	15	.....	.....	.....	.....
17	12.5	6	18	13	.....	.....	2.5	8
18	.....	.....	18	15	.....	.....	.....	.....
19	.....	.....	18	13	.....	.....	3.5	1.2
20	.....	.....	.....	.....	.....	.....	3.5	1.0
21	8	4	.....	.....	.....	.....	3.0	1.0
Average.	9.9	6.6	18.3	15.1	10.9	7.2	2.7	.8

[1] These leaves were used for the readings given in Tables I, II, and V, and each leaf has the same number in all the tables.

COMPARISON OF FACTORS FOR DIFFERENT REGIONS

A comparison of the observations of stomatal numbers and pore lengths, leaf size and maturity at different times and places and under various conditions indicates the constancy of existing relations. These studies have been made in the field in Wisconsin and Colorado and in the department greenhouse at Washington, D. C. (Table IV). In general, the sizes of leaves are not comparable as read from these three places in that the periods of observation were varied and the controlling factors were different. However, the variations in the number and size of the stomata on the different leaves in a given locality have remained uniform in all readings.

The heart leaves, as would be expected, always exhibited more stomata per unit area and had shorter pore lengths than the mature leaves on the same plant, and, in turn, the mature leaves showed more stomata per unit area than the old mature leaves. It is to be noted, however, that heart leaves in Wisconsin, although comparing them with those studied in Colorado in stomatal pore lengths, showed twice as many stomata per unit area, indicating less maturity and consequently a greater possible ultimate development in area of leaf surface. This difference probably was due in great measure to the almost constant presence of leafspot on the plants observed in Colorado and the great freedom from it in the Wisconsin field from which the data were taken. The accumulative effect of the disease on the plant would be shown by the development of smaller sized leaves with a lessened number of stomata per unit area, showing that they were maturing at a size below normal.

TABLE IV.—*Comparison of the average size of leaf, stomatal numbers, and pore lengths on different leaves of sugar-beet plants studied in Wisconsin, Colorado, and Washington, D. C.*

Locality and leaf maturity.	Size of leaf.		Number of stomata per square millimeter of leaf surface.		Stomatal pore length.		Number of leaves in averages.
	Length.	Width.	Upper.	Lower.	Upper.	Lower.	
Wisconsin:[1]	*Cm.*	*Cm.*			μ	μ	
Heart	9.9	6.6	289.8	353.5	14.0	14.0	13
Mature	18.3	15.1	100.7	130.6	28.5	27.1	16
Old mature	10.9	7.2	80.1	105.0	31.1	30.5	10
Cotyledons	2.7	.8	54.7	73.2	31.8	32.1	18
Colorado:[2]							
Old heart	10.2	12.1	144.9	206.2	.....	.....	6
Old heart, uninfected[3]	11.8	8.6	145.9	187.5	14.4	14.8	11
Young mature, infected[3]	13.5	10.3	105.9	142.8	17.8	17.6	13
Mature	16	14.4	80.4	109.6	19.4	18.1	26
Washington, D. C.:[4]							
Old heart	5.3	3.1	161.0	.....	.....	.....	18
Mature	6.7	4	98.0	.....	.....	.....	56
Old mature	6.9	4.2	74.5	.....	.....	.....	57

[1] The results given are the averages taken from Tables I, II, and III.
[2] Readings made in the field from June to August, inclusive, 1913.
[3] The results given are the averages taken from Table X.
[4] Readings made during January, 1914, on potted plants about 8 weeks old grown in the greenhouse.

Mature leaves from Colorado have approximately the same number of stomata per unit area as old mature leaves from Wisconsin, although the stomatal pore lengths are less in the former than in the latter. This would seem to be due in part to the fact that the stomata read in Colorado were not open as widely as those read in Wisconsin, and thus their maximum pore length would not be attained when observed. However, the stomata which were well open in Colorado often had a pore length equal to the average in Wisconsin. The Wisconsin records include the readings made only early in the season on one day under favorable environmental conditions when the stomata were generally wide open. On the other hand, the Colorado records include readings made on various days throughout the season and often under unfavorable environmental conditions when the stomata were only slightly open, and thus they exhibited a short pore length. In such a case the stomatal numbers offer a safer criterion of leaf maturity than the stomatal pore lengths.

The number of stomata per unit area were also read on leaves from a normal mother beet plant growing in the field at Madison, Wis., on July 30, 1914, and the results obtained were entirely comparable to those from the first-year beets, in that leaf maturity could be indicated by the same stomatal numbers. The increase in number of stomata from the oldest, or basal, leaves to those occurring near the tips of the stalks, or the younger leaves, is shown in the following tabulation:

Length of leaf.	Width of leaf.	Average number of stomata per square millimeter of upper leaf surface.	Number of readings.
Cm.	*Cm.*		
20	17	107.9	2
9	5	121.2	3
9	5	137.8	3
6	3.5	187.6	3
4.5	2	204.2	3
3	1.3	240.7	2

LEAF MATURITY AND STOMATAL MOVEMENT

Observations made at different times and on many plants have shown that the degree of stomatal movement is greatly influenced by leaf maturity. In the detailed tests reported, the readings of the stomatal pore widths on leaves of different maturities were made in the field at Madison, Wis., on a day when the sunlight was fairly strong and constant, the temperatures comparatively high, and the relative humidities above 60 per cent (fig. 1). This combination of factors was favorable for stomatal opening, as will be shown later under "Environmental factors." The leaves used in this test were the same as those from which the stomatal numbers and pore lengths have been given in Tables I, II, and III.

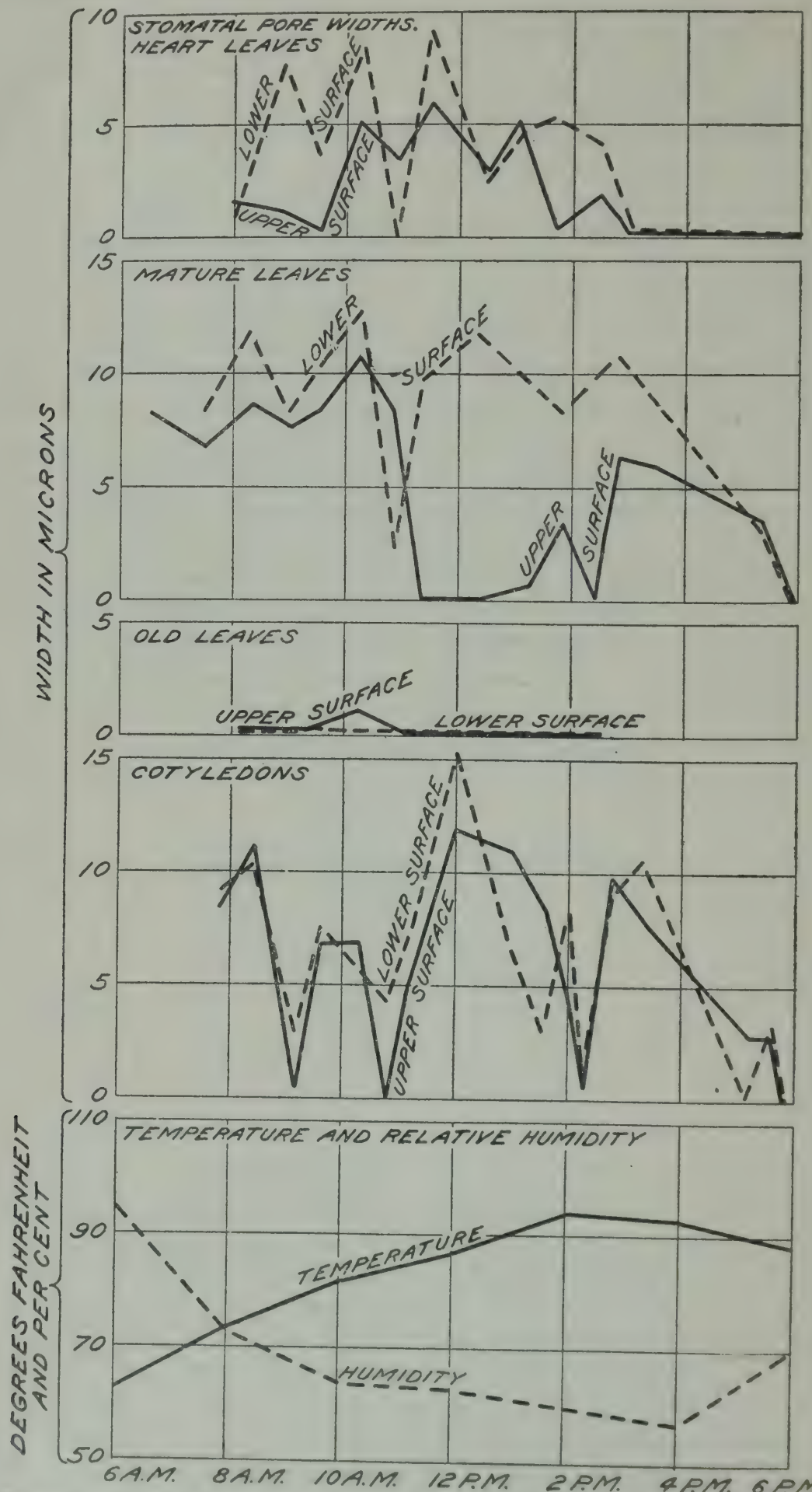

FIG. 1.—Stomatal pore widths on heart, mature, and old leaves and cotyledons of the sugar beet in the field, together with temperatures and relative humidities taken among the plants at Madison, Wis., on July 6, 1914 (Table V).

The results (Table V and fig. 1) show that the widths of the stomatal pores on cotyledons and mature leaves were greater than those on the heart leaves. In general, the stomata on the cotyledons and the lower surface of the mature leaves remained open throughout the day, while those on the heart leaves were entirely closed at 3 p. m. Those on the upper surface of the mature leaves showed a tendency to close from 11 a. m. to 1 p. m., and then to reopen before their final closure at 6 p. m. Shreve (8) found the stomata of *Parkinsonia microphylla* to exhibit this same tendency, since they closed partly during midday and reopened again during the afternoon. The stomata on the old leaves exhibited only slight movement and that on the upper leaf surface from 9 to 11 a. m. Readings were not made early enough in the day to determine the time of initial opening, but the curves indicate that the stomata on the heart leaves opened later than those on the mature leaves and cotyledons. This is shown in figure 1, in that at 8 a. m. the stomatal pore width on the heart leaves was very much less than on the mature leaves and cotyledons, being not more than 2μ on the heart leaves as compared to about 9μ on the others. On cotyledons the stomatal openings on the upper and the lower leaf surfaces remained quite comparable throughout the day. On the mature and heart leaves, however, the stomata of the lower surfaces exceeded in width of pores those of the upper surface. This relation was found to occur almost constantly throughout the day. In all cases the stomata on the upper surfaces closed at about the same time as those on the lower surfaces.

TABLE V.—*Effect of leaf maturity on average stomatal pore widths on the upper and lower leaf surfaces of the sugar beet. Readings*[1] *were taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.			Mature leaves.			Old leaves.			Cotyledons.			Time of reading.	Temperature.	Humidity.
	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.			
	a. m.	μ	μ	*a. m.*	μ	μ	*a. m.*	μ	μ	*a. m.*	μ	μ	*a. m.*	°F.	
1	.....	.....	.....	6.30	8.4 (2)	.....	.....	.....	.....	.....	.....	.....	6.00	62	95
2	.....	.....	.....	7.30	6.7 (3)	8.4 (2)	.....	.....	.....	7.50	8.4 (3)	9.2 (5)	.....	.....	.....
3	8.00	2.5 (6)	0.3 (6)	8.15	7.6 (12)	11.8 (6)	8.05	0.4 (6)	0.0 (4)	8.25	11.3 (8)	10.1 (9)	8.00	74	73
4	8.50	2.1 (8)	7.6 (8)	9.00	7.6 (7)	8.4 (3)	8.50	.4 (6)	.0 (6)	9.10	.4 (6)	2.9 (7)	.....	.....	.....
5	9.30	.1 (5)	3.8 (6)	9.35	8.4 (7)	10.5 (4)	9.25	.3 (5)	.4 (6)	9.40	6.7 (6)	7.1 (7)	.....	.....	.....
6	10.15	5.0 (5)	8.4 (4)	10.15	10.9 (7)	12.7 (5)	10.10	1.3 (8)	.0 (5)	10.20	6.7 (6)	.....	10.00	82	64
7	10.55	3.4 (7)	.0 (5)	10.50	8.4 (9)	2.5 (4)	10.55	.0 (3)	.0 (4)	10.50	.0 (5)	4.2 (8)	.....	.....	.....
8	11.30	5.9 (5)	9.2 (5)	11.20	.0 (7)	9.7 (6)	11.25	.0 (5)	.0 (5)	11.15	5.0 (7)	7.1 (6)	.....	.....	.....
	p. m.			*p. m.*			*p. m.*			*p. m.*			*p. m.*		
9	12.30	2.9 (7)	2.5 (6)	12.15	.0 (3)	11.8 (6)	12.25	.0 (6)	.0 (6)	12.00	12.7 (3)	15.2 (3)	12.00	87	63
10	1.05	5.0 (4)	4.6 (4)	1.10	.8 (6)	9.7 (4)	1.20	.0 (4)	.0 (4)	1.00	10.9 (4)	6.7 (5)	.....	.....	.....
11	1.45	.3 (5)	6.3 (4)	1.50	3.4 (12)	8.4 (5)	1.55	.0 (5)	.0 (5)	1.35	8.4 (5)	2.9 (4)	.....	.....	.....
12	2.25	1.7 (5)	4.2 (5)	2.20	.0 (5)	9.7 (6)	2.25	.0 (6)	.0 (5)	2.00	4.6 (4)	8.4 (4)	2.00	94	60
13	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.15	.3 (6)	.4 (8)	.....	.....	.....
14	2.55	.0 (5)	.2 (3)	2.50	6.3 (6)	2.6 (7)	.....	.....	.....	2.45	9.7 (5)	9.2 (6)	.....	.....	.....
15	.....	.....	.....	3.25	5.9 (6)	.....	.....	.....	.....	3.15	8.0 (6)	10.5 (6)	4.00	93	57
16	5.30	.0 (5)	.0 (5)	5.25	5.2 (8)	4.6 (10)	.....	.....	.....	5.15	2.9 (7)	.0 (4)	.....	.....	.....
17	.....	.....	.....	5.50	3.4 (9)	6.3 (9)	.....	.....	.....	5.40	2.4 (12)	3.1 (9)	.....	.....	.....
18	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.50	.0 (5)	.0 (6)	.....	.....	.....
19	6.00	.0 (5)	.0 (5)	6.00	.3 (3)	.0 (4)	.....	.....	.....	6.15	.0 (5)	.0 (5)	6.00	88	70

[1] These leaves were used for the readings given in Tables I, II, and III, and each leaf has the same number in all the tables.

This, then, would indicate that the stomata on old leaves exhibit very little movement; that those on heart leaves open, but not so widely as on mature leaves, and close earlier; that on cotyledons and mature leaves they open widely, indicating their great activity. Therefore, in the study of the environmental factors influencing stomatal movement only mature leaves have been considered, since they were always available and responded readily to changes in environment. They also represent that portion of leaf growth which is most susceptible to infection by *Cercospora beticola*. If it is true, as claimed by Iljin (4) and others, that variation in the osmotic pressure of the guard cells regulates stomatal movement, then it might be concluded that the leaves which exhibit the greatest stomatal movement are also the most active metabolically and are consequently the most important to plant development.

ENVIRONMENTAL FACTORS

It is generally agreed by various investigators that the chief external factors influencing stomatal movement are light and temperature, while a difference of opinion exists as to the influence of relative humidity. Some believe that humidity greatly affects the degree of stomatal opening, while others consider it of only minor importance. Wilson and Greenman (12) found that the stomata on plants of *Melilotus alba* which were left covered with a glass case, thus being in a nearly saturated atmosphere, were well open, while on those which were left standing in the drier open air the stomata were nearly all closed. Darwin (2) gave evidence to prove that stomata were very sensitive to changes in the humidity, closing on being taken from a high to a low humidity and opening under the reverse conditions when all the plants were exposed to approximately the same light. According to Lloyd (6) "there is a small amount of evidence that a high relative humidity favors, as a condition, the wider opening of the stomata in the ocotillo" and in regard to *Mentha piperita*, also a desert plant, he concludes ". . . in these plants, that as long as wilting does not take place a low relative humidity does not reduce the stomatal opening."

As shown by the present study, the writers believe that, while light may be considered a fundamental factor in stomatal movement, yet stomatal closure is effected by low relative humidity, even though light is active. The relative humidity present at any time, together with an optimum temperature, has been found to be a good criterion of the amount of stomatal movement that may be possible under the existing conditions.

LIGHT

In this study no attempt has been made to determine the exact relation of light to stomatal movement. Only a few scattered readings have been made to determine what effect direct sunlight has on stomatal

opening (Table VI), and the results agree, in general, with those obtained by Lloyd (6) with desert plants. When the entire leaf was exposed to sunshine, as when the leaf blade stood parallel to the sun's rays, the stomata showed the same or a greater pore opening on the lower than on the upper leaf surface (series A). This was also found to be true with leaves entirely in the shade (series B). When the sun struck vertically upon the leaf blade, an accelerating effect on stomatal opening usually resulted, regardless of which surface was exposed to the sun (series C and D). This is also in agreement with the work of Balls (1, p. 231), in which he found that the stomata on the cotton plant opened widely in the sunlight and closed partly in the shade. The leaves in series C, read on July 18, indicate a point noticed by Lloyd (7) that the stomata near the apex of a leaf might have less pore width than those near the base, "a condition readily understandable if wilting is progressive from the apex of the leaf downward."

TABLE VI.—*Effect of sunshine and shade on the width of the stomatal pore opening of the sugar-beet plant at Rocky Ford, Colo., in 1913*

Date.	Hour.	Relative humidity.	Temperature.	Stomatal pore width.	
				Upper surface.	Lower surface.
SERIES A (ENTIRE LEAF IN SUN)					
			°F.	μ	μ
May 17	7.15 a. m.	58	67	1.8 (3)	1.8 (4)
Aug. 4	7.30 a. m.	77	68	6 (6)	[a] 7.8 (7)
SERIES B (ENTIRE LEAF IN SHADE)					
May 17	7.15 a. m.	58	67	0 (6)	0 (6)
June 2	7.45 a. m.	100	65	1.5 (8)	[b] 6.3 (8)
June 3	9.30 a. m.	71	69	0	0
Aug. 4	7.30 a. m.	77	68	1.3 (7)	4.7 (5)
SERIES C (UPPER LEAF SURFACE IN SUN; LOWER IN SHADE)					
May 24	7.30 a. m.	69	68	4.2 (4)	8.6 (5)
May 26	1.45 p. m.	51	91	9.4 (6)	7.6 (4)
May 27	8.00 a. m.	62	78	4.5 (4)	0 (6)
July 18	9.00 a. m.	91	72	[c] 5.7 (3) [d] 7.2 (3)	[c] 5.7 (3) [d] 6.5 (3)
Aug. 4	7.30 a. m.	77	68	5.7 (4)	0 (5)
SERIES D (UPPER LEAF SURFACE IN SHADE; LOWER IN SUN)					
May 19	8.00 a. m.	65	63	[e] 3.8 (7)	[f] 10.8 (6)
May 24	7.30 a. m.	69	68	2.5 (5)	3.4 (5)
May 26	1.45 p. m.	51	91	6.5 (6)	9.4 (5)
May 27	8.00 a. m.	62	78	1.0 (6)	7.4 (6)

[a] Wet from dew.
[b] All wide open.
[c] Apex.
[d] Base.
[e] Many closed.
[f] All open.

TEMPERATURE AND RELATIVE HUMIDITY

The determination of the effect of varied temperature and relative humidity on the opening of the stomatal pore of the sugar-beet plant was made under conditions which were somewhat under control. The plants used for study were first-year beets about 3 months old and of thrifty growth which had been grown in a deep soil bed in the greenhouse at Rocky Ford, Colo. A good root development was thus made possible, and normal leaf production had been accomplished. The leaves used for the readings were all mature and averaged about 14 cm. wide and 20 cm. long. Direct readings of the widths of the stomatal pores were made on plants both left free in the greenhouse and kept covered during the time of the experiment with a large glass humidity box (Pl. LXXX, fig. 2) of about 20 cubic feet capacity. This box was five-sided and could be placed over plants in a manner comparable to the bell-jar method. Aeration was made possible by this means and room was also available for a hygrothermograph, so that constant-humidity and temperature records were available without any disturbance of the plants. Comparable hygrothermograph records were also kept among the leaves freely exposed in the greenhouse and both instruments were checked by means of a cog psychrometer (Pl. LXXX, fig. 2). Middle-blade portions of different leaves were taken from all plants and stomatal readings made by the "in situ" method. The definite data of the experiments conducted on May 16, 17, and 20 and June 3 are given in Table VII and the graphic representations in figures 2 to 5.

TABLE VII.—*Effect of varied temperature and relative humidity on stomatal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913. Comparable readings were taken in the greenhouse on plants covered by a large glass humidity box and on those left freely exposed to ordinary greenhouse conditions*

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths.[a]		Temperature.	Relative humidity.	Average stomatal pore widths.[a]	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
May 16:[b]	° F.	Per ct.	μ	μ	° F.	Per ct.	μ	μ
9.00 a. m.	68	70	9.0 (5)	7.9 (5)	77	43	1.8 (3)	0 (4)
1.30 p. m.	92	46	12.6 (4)	10 (4)	90	16	0 (5)	0 (5)
4.15 p. m.	89	54	8.6 (5)	0 (4)	93	18	0 (5)	0 (5)
7.00 p. m.	71	79	0 (5)	.36 (4)	75	24.5	0 (7)	0 (5)
May 17:[c]								
5.00 a. m.	51	95	.36 (6)	2.1 (12)	52	73.5	0 (5)	0 (5)
7.15 a. m.	60	67	6.8 (6)	2.7 (4)	67	58	1.8 (3)	1.8 (5)
8.30 a. m.	63	66	7.3 (7)	8.2 (9)	71	50	2.5 (9)	2.1 (4)
10.00 a. m.	73	65	6.8 (4)	7.5 (4)	78	38	5.4 (4)	5.4 (5)
11.00 a. m.	80	63	7.2 (6)	9 (5)	83	31	1.8 (6)	5.4 (5)
1.30 p. m.	79	60	7.2 (4)	7.2 (4)	80	32	7.2 (3)	6.8 (4)
4.20 p. m.	70	74	7.2 (3)	6.4 (3)	71	34	0 (4)	0 (5)

[a] The number of readings is given in parentheses following each average.
[b] The sun shone brightly throughout the entire day.
[c] The sun shone brightly up to 4 p. m.

TABLE VII.—*Effect of varied temperature and relative humidity on stomal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913*—Continued

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths.		Temperature.	Relative humidity.	Average stomatal pore widths.	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
May 20:[a]	°F.	Per ct.	μ	μ	°F.	Per ct.	μ	μ
5.00 a. m.	50	95	0.3 (6)	0.4 (13)	51.5	93	0 (7)	0.3 (9)
6.00 a. m.	51	95	.7 (8)	1.8 (9)	52	91	0.25 (11)	.2 (10)
7.00 a. m.	53	94	.3 (7)	1.6 (7)	54	83	.14 (10)	.28 (10)
8.00 a. m.	56	85	3.24 (7)	5 (6)	61	65	2.8 (6)	1.4 (8)
8.30 a. m.	63	76	2.16 (6)	2.8 (6)	63	64	2.1 (8)	2.1 (7)
9.00 a. m.	64	75	5.7 (6)	7.2 (5)	65	59	3.9 (6)	4.3 (6)
9.30 a. m.	64	75	6.1 (9)	7.2 (9)	65	59	4.6 (7)	3.8 (6)
10.30 a. m.	65	66	5.7 (6)	5.7 (4)	67	53	5 (6)	7.5 (7)
11.00 a. m.	68	65	7.2 (6)	7.2 (6)	68	57	1.8 (6)	1.4 (7)
11.45 a. m.	71	66	7.2 (4)	7.2 (4)	72	53	2.1 (6)	0 (5)
1.30 p. m.	75	57	9 (4)	11 (5)	74	45	1.08 (5)	1.08 (6)
2.15 p. m.	75	58	7.2 (6)	7.5 (5)	74	42	3.2 (7)	1.6 (6)
2.45 p. m.	73	57	7.2 (5)	7.2 (4)	71	42	3.6 (7)	4.06 (6)
3.30 p. m.	74	53	5.7 (5)	3.2 (5)	71	42	1.5 (5)	0 (5)
4.00 p. m.	75	53	6.8 (5)	6.1 (6)	74	40	2.1 (5)	0 (5)
June 3:								
7.45 a. m.	67	100	3.6 (3)	4.3 (4)	69	69	1.8 (4)	1.08 (4)
9.00 a. m.	70	100	6.3 (4)	7.38 (4)	67	64	2.5 (5)	.14 (5)
9.30 a. m.	72	100	7.5 (4)	7.2 (4)	69	71	2.8 (4)	0 (5)
10.00 a. m.	74	100	7.5 (4)	7.4 (4)	75	68	4.4 (5)	2.5 (5)
10.15 a. m.	80	100	7.2 (4)	7.3 (5)	73	75	6.1 (5)	6.1 (5)
10.30 a. m.	82	100	6.4 (4)	5.8 (4)	73	67	7.2 (4)	6.8 (5)
11.45 a. m.	93	100	9.3 (4)	9.3 (3)	79	62	4.5 (4)	9.1 (5)
12.15 a. m.	94	97	7.8 (4)	8.5 (4)	82	63	9.4 (3)	8.1 (3)
1.30 p. m.	96	93	7.02 (6)	7.5 (5)	80	56	3.8 (10)	2.7 (9)
2.00 p. m.	94	95	9.4 (6)	9.4 (6)	80	58.5	1.6 (11)	2.3 (12)
2 30 p. m.	85	95	7.8 (5)	7.8 (4)	75	57	5.8 (6)	0 (8)
3.00 p. m.	89	100	5.4 (6)	6.4 (6)	75	57	0 (5)	0 (5)
3.30 p. m.	75	100	5.4 (6)	4.3 (5)	75	57	0 (5)	0 (6)

[a] Intermittent clouds and sunshine up to 11.45 a. m., then bright sunshine until 2.25 p. m.; cloudy to 3.30 p. m., and then sunshine for the rest of the day.

Usually the temperature in the humidity box was practically the same as that outside in the greenhouse at the same time. Although no definite study has been made to determine the temperature most favorable to stomatal movement, it is to be noted that good stomatal opening occurred between 8 a. m. and 5 p. m., and during that time the temperature increased, on the average, from about 65° to 85° F. and decreased to 80° F. Only on June 3 was the temperature in the humidity box much higher than that outside in the greenhouse, and it appears that neither of these temperatures (96° in the humidity box and 80° in the greenhouse at 1.30 p. m.) produced a change in the degree of stomatal opening.

On the other hand, the humidity in the two places was quite different, being always higher inside than outside of the humidity box. To this difference in humidity the marked variation in the pore opening of the stomata has been attributed. For example, on May 16 the humidity ranged about 30 units higher inside than outside of the box (fig. 2), and the stomata were well open in the former place and closed practically throughout the day in the latter. On the upper leaf surface in the greenhouse only slight opening occurred at 9 a. m. and this disappeared

by 1.30 p. m. During this time the relative humidity fell from 43 to 16 while inside the humidity box it ranged from 70 to 46 and the average width of the pores of the stomata increased from 9 to 12.6μ. The stomata on the upper leaf surface were also open wider and remained open longer than those on the lower, while all were closed by 7 p. m. These points

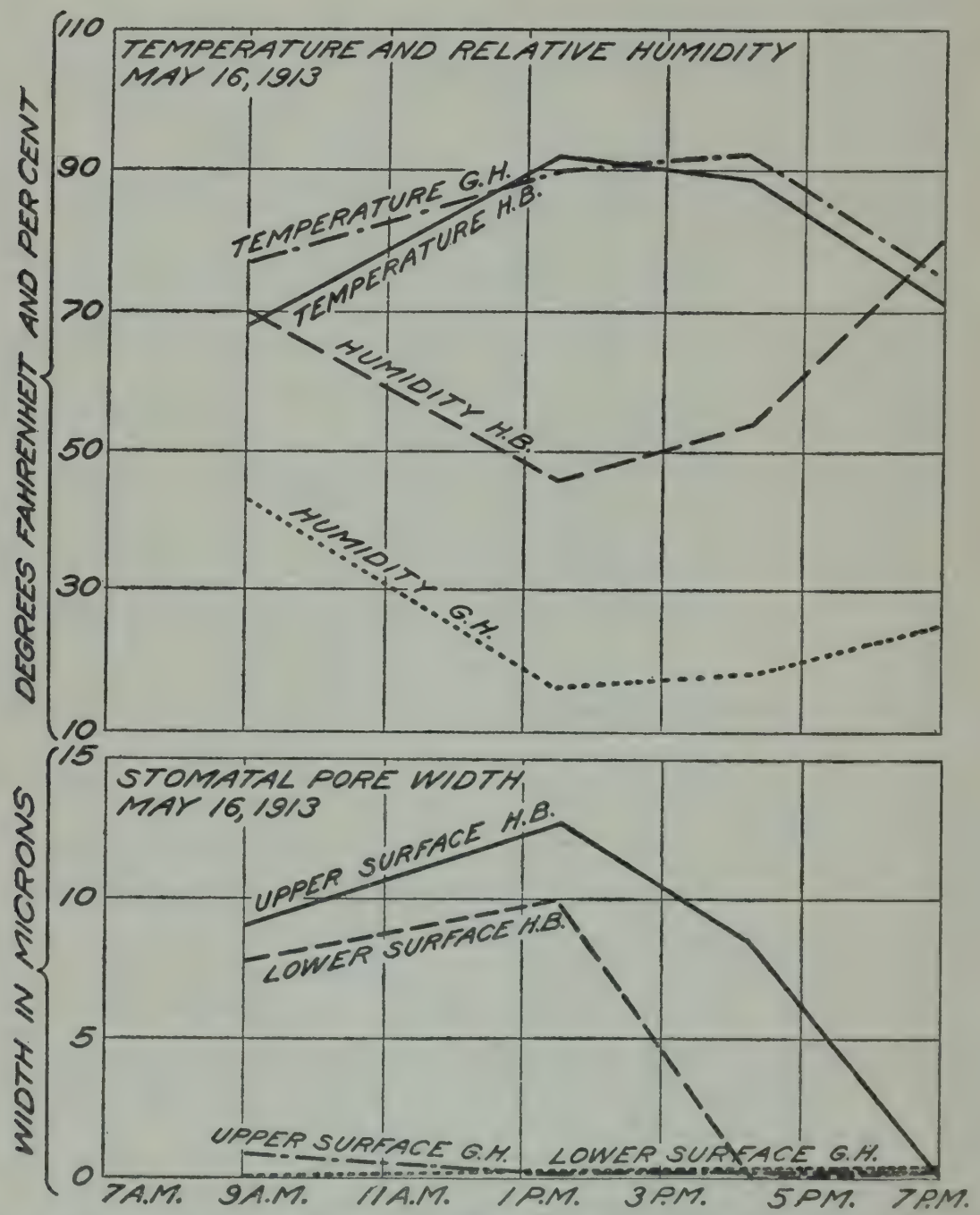

FIG. 2.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 16, 1913 (Table VII).

seem to indicate that the relative humidity as supported by soil moisture, transpiration, etc., must remain, in general, above a certain percentage in order that the maximum influence of light may be realized. Otherwise, if the humidity is too low, the light factor becomes in some way less operative, and the stomata open to a less extent and close earlier.

In another test made on the following day, the humidity ranged from 9 to 40 units higher inside the humidity box than in the greenhouse (fig. 3), and throughout the day the stomata were open wider in the former place than in the latter. At 5 a. m. all the stomata were closed except those on the lower leaf surface in the humidity box, which were slightly open. In general, the initial opening probably occurred soon after 5 a. m., for at 7.15 a. m. the stomata were all open, those in the humidity box being open wider than those outside. This point opposes the theory that the stomata in the humidity box remain well open during midday on account of the less intense light due to the additional window-

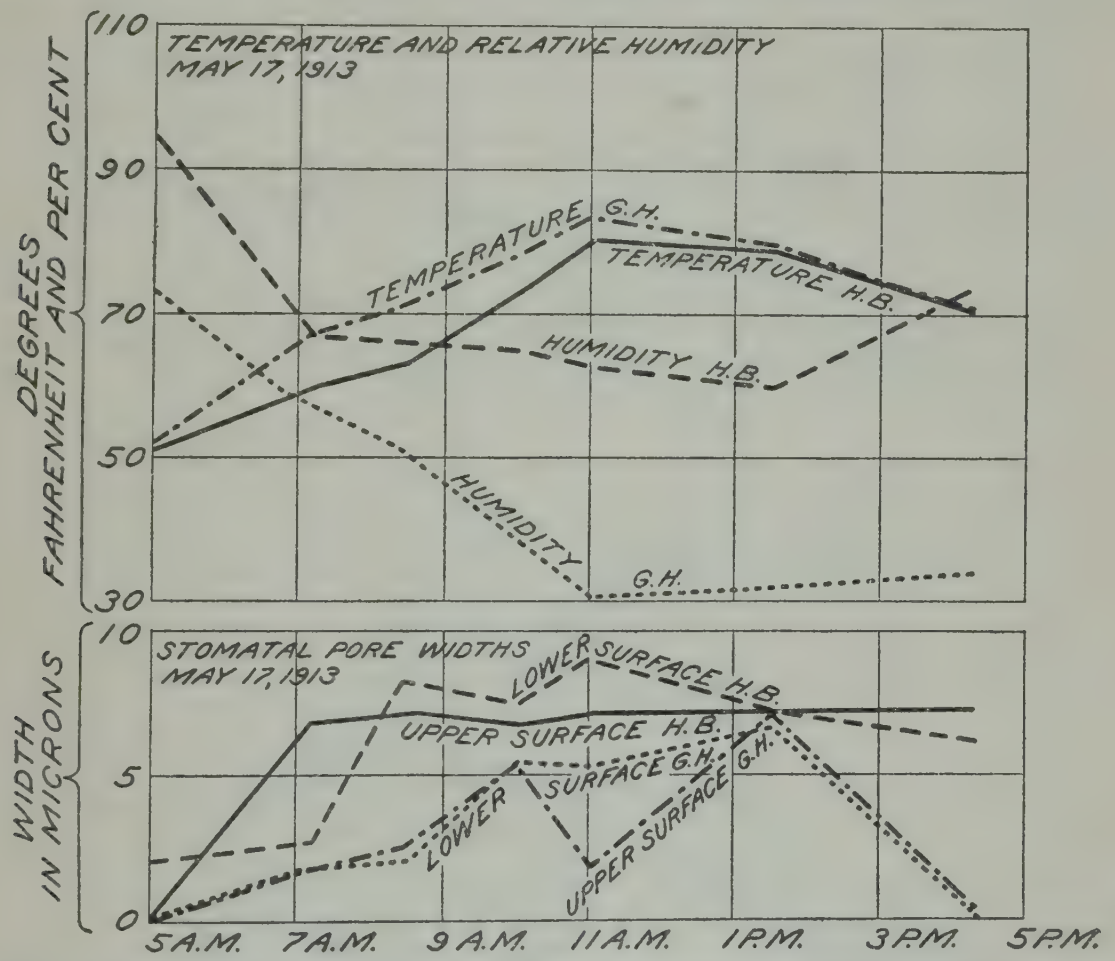

FIG. 3.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 17, 1913 (Table VII).

glass covering, while during the same period, those outside the humidity box close as a reaction to the more intense unobstructed light. If this were true, then, the stomata in the humidity box would open later in the day than those outside, because the light in the former place would be weaker. As a matter of fact, the stomata in the humidity box opened earlier and had greater pore width than those outside, even when thus exposed to the weaker light. The conclusion that may be drawn from this is that the relative humidity is the indicative factor of the causes which produce this difference. It should be noted that in the humidity box the humidity did not fall below 60 during the day, and the stomata were still open at 4.20 p. m., when the last reading for the day was made.

Outside in the greenhouse the humidity ranged from 31 to 34 after 11 a. m., and the stomata were entirely closed at 4.20 p. m.

A comparison of the stomatal pore widths of the leaves in the greenhouse on May 16 with those in the same place on May 17 shows that on the former day the stomata were practically closed all day, while on the latter they opened early and remained fairly well open till after 2 p. m. The humidity on the two days was quite different, being appreciably higher on the 17th than on the 16th. This offers an explanation for the differ-

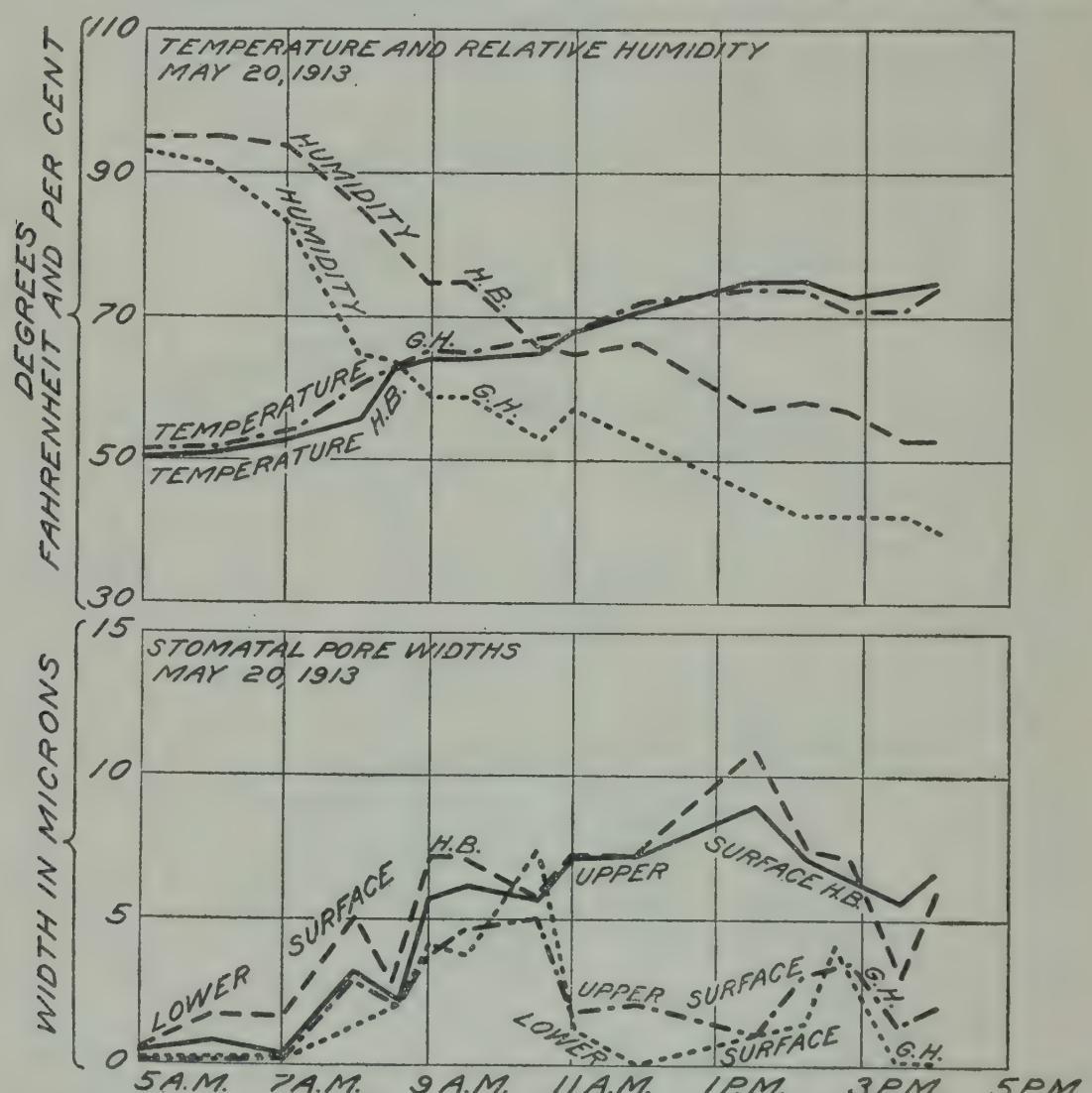

FIG. 4.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 20, 1913 (Table VII).

ence in stomatal pore opening, though, of course, conditions on the two separate days can not be compared too closely.

In another test, made on May 20, the stomata in the humidity box again showed greater widths of pores than those outside in the greenhouse (fig. 4) and the humidity ranged about 10 units higher throughout the day in the former place than in the latter. The greatest difference in the stomatal opening in the two places occurred after 11 a. m. when the stomata in the humidity box had much greater stomatal pore widths than those outside. The humidity remained generally near or above 60 in the box, while outside it was, on the average, below 50. The initial

opening in both places occurred about 5 a. m., and in the humidity box the opening on the lower leaf surface exceeded that on the upper, this relation remaining uniform throughout the day. This tendency is also indicated in figure 3 in the greater stomatal opening of the lower over the upper leaf surface in the humidity box. These observations in general agree with the findings of other investigators. Darwin (2) found that the stomata on the lower surface often opened earlier and remained open longer than those on the upper, though this was not always true. He believed that the difference in the opening was due to illumination rather than to any inherent distinction between the stomata. Livingston and Estabrook (5) found in the study of the stomata on several different

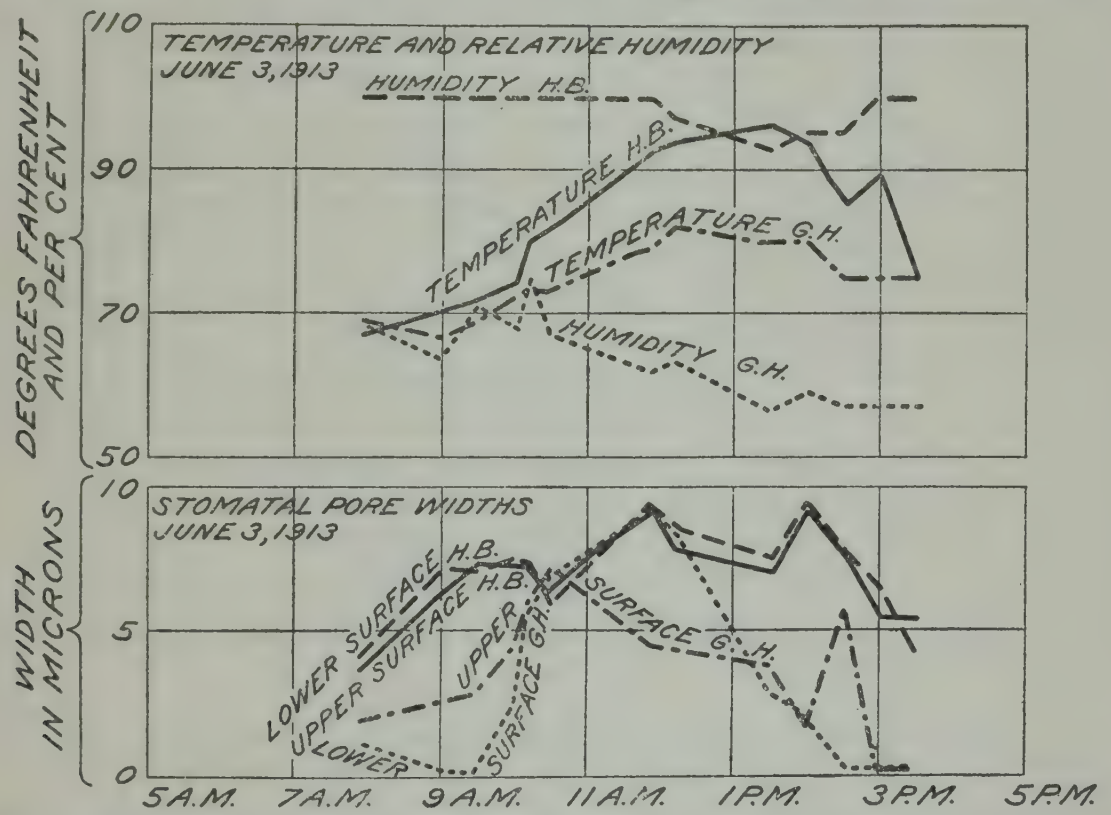

FIG. 5.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on June 3, 1913 (Table VII).

kinds of plants that those on the upper surface open and close more rapidly and close more completely than those on the lower. Lloyd (7) observed with cotton that—

> The initial opening on September 30, 1911, occurred about 6.30 a. m., from which hour on a progressive opening movement was followed, the stomata of the lower surfaces opening somewhat in advance of those of the upper, though some exceptions to this appear.

Again, on June 3, after all the beds in the greenhouse had been watered on the preceding evening and the humidity box placed at that time over a portion of the plants for the test, the same general results were obtained, in that the stomata opened wider and remained open longer in the humidity box with higher humidity active for a longer period than in the green house (fig. 5). During this test the stomata in the greenhouse remained open during midday till about 3 p. m., owing probably to the fact that

the humidity remained comparatively high—above 60. A comparable difference is noted in the humidities and stomatal pore widths taken on this date and on May 20. After 11 a. m. the humidity on June 3 was generally above 60 and the stomata had pore widths of more than 5μ until after 1 p. m., when the opening gradually decreased until closure occurred about 3 p. m. On May 20, after 11 a. m., the humidity was generally slightly above 50 and the stomatal pore opening was reduced from 5μ at 10 a. m. to about 2μ at 11 a. m., after which time it seldom exceeded this amount.

A few readings were made in the field at various times during the season to get an indication of the stomatal movement under such conditions. On June 21 the stomata were found to be well open at 3 p. m. and later at a humidity of 60 or above (Table VIII). On June 23 the stomata were widely open from 8.30 to 10.40 a. m., even though the humidity dropped to as low as 40 at 10.10 a. m. The readings were not continued long enough to determine whether this low humidity would produce stomatal closure during midday. However, the readings taken on July 18 indicate that at 2 p. m. the stomata had a smaller pore width than at any other reading during the day and at that time the lowest humidity (57.5) of the day occurred. Two readings were made at the same time in this field. The one made near the center of the field, where the plants were large and close together, showed the stomata to be open (8.7 upper, 1.8 lower) at a humidity of 57.5, while the other made at the edge of the field, where the plants were small and far apart, showed the stomata to be closed at a humidity of 43.5. The maturity was determined to be the same for both sets of leaves used. In this case the soil-moisture content was noted to be much lower at the edge than in the center of the field, as the low humidity would indicate.

TABLE VIII.—*Stomatal pore openings on leaves of sugar-beet plants growing in the field at Rocky Ford, Colo., in 1913, together with the temperature and relative-humidity records taken among the leaves at that time*

Date and time of readings.	Temperature.	Humidity.	Average stomatal pore widths.[1]	
			Upper leaf surface.	Lower leaf surface.
June 21:	°F.		μ	μ
3.00 p. m.	85	60	10.4 (4)	10.08 (5)
3.45 p. m.	.....	.....	4.96 (7)	6.6 (6)
4.30 p. m.	77	65	1.72 (9)	5.1 (7)
June 23:				
8.30 a. m.	74	60	10.8 (3)	9.9 (4)
9.20 a. m.	79	52	13.5 (4)	10.3 (4)
10.10 a. m.	83	39.5	10.6 (7)	7.1 (8)
10.40 a. m.	85	46.5	12.9 (5)	10.8 (3)
July 18:				
9.00 a. m.	72	91	6.3 (6)	6.4 (3)
10.30 a. m.	74	87	4.6 (3)	4.1 (3)
11.15 a. m.	82	67	9 (5)	14.4 (4)
2.00 p. m.[2]	83	57.5	8.7 (8)	1.8 (10)
2.00 p. m.[2]	89	43.5	0 (10)	0 (10)

[1] The number of readings made is given in parentheses following each average.
[2] These readings were taken at two different places in the same field.

Therefore, it may be concluded that if the relative humidity remains above 60 during the hours of daylight the stomata will probably be found open, while with a lower humidity the stomatal opening will decrease until it becomes greatly reduced and with still lower humidity the stomata may usually be found completely closed, or at least as nearly so as ever occurs. In an irrigated area especially, where the humidity is very largely controlled by the soil moisture, a high humidity may be directly due to a high soil-moisture content and would indicate increased plant activity. The beneficial effects of high humidity on increased plant growth is generally recognized. Wollny (13), who grew plants of barley, vetch, alfalfa, flax, and potato under conditions giving three degrees of humidity, found that with an increase in the degree of humidity there was an increase in the production both of the absolute quantity of fresh material and of dry matter. On the other hand, low soil-moisture content would greatly check such activities, and a low humidity, which would be associated with such a condition, would indicate marked differences in stomatal movement. Thus, it appears that a low humidity with its associated causes and effects results in diminished stomatal movement, and then the existing percentage of relative humidity becomes an important and convenient index to stomatal activities.

FACTORS INFLUENCING INFECTION

A consideration of the factors additional to, and somewhat preliminary to, stomatal movement that have been found to influence infection includes some of the conditions that affect both parasite and host in this relation. The effect that media, light, and temperature have on the rapidity of germ-tube growth becomes important in the relation that the fungus bears to leaf penetration. On the other hand, the maturity of the leaf, which controls stomatal mobility, plays a comparable part in this interrelation.

RAPIDITY OF GERM-TUBE GROWTH

No difference has been found to exist in the effect that north light and darkness have on the rapidity of germ-tube growth at a constant temperature. From the data given in Table IX it appears that all conidia germinated and had approximately the same average germ-tube lengths, together with a comparable average number of germinating cells per spore, regardless of the light factor. Consequently, under field conditions conidial germination would be expected to proceed equally fast under night or day conditions, except in direct sunlight, where the heat factor becomes important in causing rapid evaporation.

TABLE IX.—*Effect of light and medium on the germination of conidia of Cercospora beticola, at a temperature of 24° C., on August 12, 1913, at Rocky Ford, Colo.*

Environment.	Number of hours of growth.	Average percentage of germinating conidia.	Average number of cells per conidium.	Average number of germinating cells per conidium.	Average length of germinating tube.
					μ
Distilled water, north light	6¼	100	.....	2.47	43.28
Distilled water, dark room	6½	100	.....	2.4	41.11
Distilled water, north light	8	100	9.42	4.14	56.31
Distilled water, dark room	8½	100	8.69	3.46	65.77
Bean decoction, north light	9	100	9.44	3.33	55.48
Irrigation water, north light	9¾	100	10.16	3.83	91.69
Soil decoction, north light	10	100	6	3.00	98.42

Germination also occurred equally well in distilled water, bean decoction, soil decoction, and irrigation water, showing that a nutrient medium did not hasten germination nor did it retard it. It is also to be noted that the conidia were incubated nearly twice as long in soil decoction as in distilled water, which would account for the longer germ tubes in the soil decoction. In both solutions 100 per cent of the conidia germinated. The condensed moisture that may be found on leaves then would seem to give a favorable medium for conidial germination and that germ-tube growth could take place rapidly in it. It has been found that only a short time is necessary for germination to take place, since newly formed conidia may begin to germinate in three hours after being placed in water cultures at 26° C. The germinating tubes from such conidia may increase 5μ in length in 40 minutes.

The effect of high temperatures on conidial germination is not considered in this discussion. However, in another phase[1] of the study of the sugar-beet leafspot, it has been determined that a period of days with extreme high night (70° F.) and day (104° F.) temperatures together with low relative humidity, a condition that may occur at times in an irrigated region, is inimical to the life of the conidia. This factor then becomes of importance in considering conidial growth and development under natural environment.

LEAF MATURITY

Near the middle of the summer or later, in a sugar-beet field infected generally with leafspot, the individual plant presents a typical picture of the disease. A cluster of uninfected heart and slightly infected young mature leaves occurs at the center of the plant, while all other leaves on the same plant are heavily infected. A comparison was made of the stomata on such heart and young mature leaves, or the oldest uninfected and the youngest infected leaves, on each of several plants. The study

[1] The thermal relations of the fungus will be discussed in a later paper entitled "Relation of climatic conditions to infection by Cercospora beticola."

was carried on in August, 1913, near Rocky Ford, Colo., and the readings of the two types of leaves from the same plant were made near together so that all time factors might, so far as possible, be eliminated. The results show that on the average the number of stomata is less and their pore length is greater (Table X) on the infected leaves than on the uninfected, showing the greater maturity of the former. Some variations in these numbers occur, but it is to be noted that the four infected leaves with the greatest number of spots present have, on the average, fewer stomata per square millimeter of leaf surface and a greater stomatal pore length than the four infected leaves with the least number of spots.

TABLE X.—*Comparative average maturity of Cercospora beticola infected (young mature) and uninfected leaves (heart) of the sugar-beet plant as shown by the number and pore length of the stomata. Readings[1] taken on August 5 to 11, 1913, at Rocky Ford, Colo.*

INFECTED YOUNG MATURE LEAVES[2]

Leaf No.	Size of leaf.		Average number of stomata.		Average stomatal pore lengths.		Number of leaf-spots per leaf.
	Length.	Width.	Upper leaf surface.	Lower leaf surface.	Upper leaf surface.	Lower leaf surface.	
	Cm.	*Cm.*			μ	μ	
1	17.5	12.5	98.4 (3)	123 (1)	19 (3)	19 (2)	24
2	17	12.5	68.06(3)	106.6 (3)	19 (5)	19 (3)	21
3	9.5	9	95.1 (3)	111.5 (3)	19 (6)	19 (3)	21
4	14.5	10	102.5 (4)	127.9 (3)	19 (4)	19 (2)	14
5	10	7.5	106.6 (3)	155.8 (2)	19 (6)	17.5 (5)	9
6	10.5	9	110.7 (2)	139.4 (2)	19 (5)	19 (5)	5
7	15	10.5	77.9 (2)	123 (2)	15.2 (6)	19 (6)	5
8	11.5	12	114.8 (2)	137.3 (4)	17.1 (6)	15.2 (2)	3
9	15	12.5	118.9 (2)	164 (2)	15.5 (5)	13.3 (2)	3
10	10.5	8.5	114.8 (2)	172.2 (2)	19 (4)	19.7 (5)	1
11	13.5	9.5	133.9 (3)	183.1 (3)	15.2 (4)	16.7 (7)	1
Average.	13.1	10.3	103.8	140.3	17.8	17.8	.....

UNINFECTED HEART LEAVES[2]

1	14.5	9.5	123 (2)	164 (1)	19 (3)	17.4 (3)	.....
2	14	9.5	118.9 (2)	172.2 (1)	15.9 (5)	15.2 (5)	.....
3	8.5	8	145.2 (3)	147.6 (3)	17.1 (8)	15.2 (5)	.....
4	12.5	8	135.3 (2)	166.4 (3)	13.1 (4)	15.2 (2)	.....
5	13	8	133.9 (3)	184.5 (2)	13.6 (6)	15.2 (4)	.....
6	10.5	7	144.3 (3)	174.6 (3)	15.2 (3)	17.1 (2)	.....
7	13	9.5	131.2 (1)	205 (2)	11.4 (4)	13.3 (6)	.....
8	9	9.5	127.1 (2)	184.4 (2)	13.9 (5)	11.4 (4)	.....
9	13	10	192.7 (2)	225 (2)	13.3 (8)	13.3 (6)	.....
10	9	6.5	192.7 (2)	241.9 (2)	13.3 (4)	15.2 (6)	.....
11	12.5	9.5	161.2 (3)	196.8 (3)	12.1 (7)	14.4 (5)	.....
Average.	11.8	8.6	145.9	187.5	14.4	14.8	.....

[1] The number of readings made per leaf is given in parentheses following each average.

[2] Infected leaf 1 was on the same plant as uninfected leaf 1, infected leaf 2 was on the same plant as uninfected leaf 2, and so on through the series. The leaves of each pair were read at the same time.

The averages for the eight leaves mentioned are:

Leaf No.	Size of leaves.		Number of stomata.		Length of stomatal pores.		Number of leaf-spots.
	Length.	Width.	Upper.	Lower.	Upper.	Lower.	
	Cm.	*Cm.*			*Cm.*	*Cm.*	
1 to 4	14.6	11	91	117.2	19	19	20
8 to 11	12.6	10.6	120.6	164.1	16.7	16.2	2

It is also to be noted that infected leaf 11, which had only one spot, had the shortest average stomatal pore lengths (except leaf 9) and the highest number of stomata per area of any of the infected leaves studied. From these figures it would further appear that of all the uninfected leaves studied, only leaf 1 would have a stomatal count and pore length that would indicate leaf susceptibility. It might be concluded that this leaf remained uninfected merely by chance and that the others were uninfected because they had not as yet reached the maturity which would allow infection to occur.

Detailed field observations made of the amount of infection that appeared on the different leaves of many sugar-beet plants during an entire season have again shown that the greatest number of leafspots developed on the mature leaves. The records from one plant are shown in Table XI. The leaves were tagged and numbered consecutively, beginning with the outermost, or oldest, so that the new leaves tagged on all days after the first one were heart leaves. As these grew older they became susceptible to leafspot, and with increased maturity usually became heavily infected, and finally the death of the leaf occurred. Those leaves, whose numbers are in italic, on the last date reported were killed by the fungus. From 400 to 1,000 spots were sufficient to kill a leaf, depending on its size, in a few days. While the death of many of the leaves not reported as killed by *Cercospora beticola* was no doubt hastened by the presence of the fungus, yet age and other factors were predominating causes of the death of the leaf.

The results obtained show that, as a rule, infection did not take place readily on old yellow leaves, but occurred most readily on active green leaves. It is true that there was often a large increase in the number of spots present on the leaves during the few days just previous to the death of the leaf, as is shown by leaves 21, 24, 25, 27, 35, and others on this one plant (Table XI), but such leaves were not normally old. They were no doubt green and quite active when infection took place and merely died prematurely and very suddenly as a result of the great number of spots produced.

TABLE XI.—*Number of leafspots present on different leaves of varying maturity on one sugar-beet plant in a medium-early field from June 24 to Sept. 19, 1913, at Rocky Ford, Colo.*

Leaf No.	June.		July.												August.												September.								
	24	28	2	7	9	10	12	14	16	21	23	25	28	30	1	5	7	9	11	13	15	18	22	25	28	30	1	3	6	8	10	13	15	17	19
1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
6	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
7	1	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
8	0	0	0	0	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9	0	0	0	0	0	0	0	2	2	2	2	3	3	3	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10	0	0	1	4	5	5	5	5	5	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11	0	0	0	0	3	3	3	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12	0	0	0	4	4	4	4	4	4	4	4	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13	0	0	2	3	5	5	5	5	5	5	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
14	...	0	0	0	0	0	0	0	0	2	2	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
15	...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16	...	...	0	0	0	0	0	0	0	0	0	0	0	4	4	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
17	...	...	0	0	0	0	0	0	0	0	1	1	3	3	8	13	28	38	38	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18	...	...	...	0	0	0	0	0	0	0	0	0	0	1	1	11	24	26	26	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
19	...	...	...	0	0	0	0	0	0	0	0	0	2	4	8	21	21	26	26	38	42	50	50	70	300	300	300	...	...	...	...	...	...	...	...
20	...	...	...	0	0	0	0	0	0	0	0	0	0	0	4	6	11	11	15	20	30	40	40	56	350	350	350	...	...	...	...	...	...	...	...
21	...	...	...	...	0	0	0	0	0	0	0	0	3	3	3	13	13	25	25	27	60	76	115	155	*450*	...	...	...	...	...	...	...	...	...	...
22	...	...	...	...	...	0	0	0	0	0	0	0	3	3	3	9	16	20	29	31	31	40	61	85	140	140	140	...	...	...	...	...	...	...	...
23	...	...	...	...	...	...	0	0	0	0	0	0	2	7	12	32	39	50	50	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
24	...	...	...	...	...	...	...	0	0	0	0	0	3	4	5	15	18	18	19	32	49	54	96	150	300	300	*400*	...	...	...	...	...	...	...	...
25	...	...	...	...	...	...	...	0	0	0	0	0	0	0	4	12	15	20	20	26	32	33	46	130	600	600	800	*800*	...	...	...	...	...	...	...
26	...	...	...	...	...	...	...	...	0	0	0	0	0	1	2	7	12	25	28	37	43	56	68	110	380	600	600	*600*	...	...	...	...	...	...	...
27	...	...	...	...	...	...	...	...	0	0	0	0	1	1	3	6	9	17	18	19	36	46	70	85	200	300	450	*500*	...	...	...	...	...	...	...
28	...	...	...	...	...	...	...	...	...	0	0	0	1	6	9	15	17	23	29	49	62	80	100	150	350	600	*600*	...	...	...	...	...	...	...	...
29	...	...	...	...	...	...	...	...	...	0	0	0	5	9	14	20	24	24	24	26	61	70	100	170	300	300	...	...	...	...	...	...	...	...	...
30	...	...	...	...	...	...	...	...	...	0	0	0	1	1	4	7	10	18	20	22	50	50	50	70	600	600	600	*600*	...	...	...	...	...	...	...
31	...	...	...	...	...	...	...	...	...	0	0	0	3	3	4	11	13	18	23	45	48	50	95	130	350	450	450	*450*	...	...	...	...	...	...	...
32	...	...	...	...	...	...	...	...	...	...	0	0	0	0	4	7	9	13	13	13	20	20	38	60	260	480	650	700	800	800	*800*	...	...	...	...
33	...	...	...	...	...	...	...	...	...	...	0	0	3	3	7	14	19	21	24	47	76	105	220	220	450	500	600	600	*600*	...	...	...	...	...	...
34	...	...	...	...	...	...	...	...	...	...	...	0	0	0	2	10	16	22	22	30	33	34	55	90	350	480	700	750	750	750	*750*	...	...	...	...
35	...	...	...	...	...	...	...	...	...	...	...	0	0	0	5	10	10	14	14	38	52	95	160	165	300	300	350	*450*	...	...	...	...	...	...	...
36	...	...	...	...	...	...	...	...	...	...	...	...	0	0	1	7	9	10	16	71	116	165	250	250	350	350	520	520	*520*	...	...	...	...	...	...
37	...	...	...	...	...	...	...	...	...	...	...	...	0	0	3	9	9	11	16	22	37	39	53	58	120	180	350	450	450	550	600	600	*600*	...	...
38	...	...	...	...	...	...	...	...	...	...	...	...	0	0	1	1	1	11	28	58	82	95	121	160	270	400	500	550	600	650	700	900	900	*900*	...
39	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	1	4	21	49	90	165	180	500	500	500	500	700	900	900	*900*	...	...	...
40	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	2	3	11	24	64	112	145	145	230	390	570	570	570	*570*	...	...	...	...
41	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	8	14	22	33	44	64	95	380	450	500	500	550	550	550	550	*550*	...	...
42	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	3	3	5	9	16	28	160	300	320	320	320	350	400	500	500	*500*	...
43	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	1	13	20	47	112	150	190	260	320	365	600	600	600	600	*600*	...	...
44	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	1	7	18	41	170	170	245	245	260	260	260	260	300	300	450
45	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	13	55	76	240	250	650	650	650	650	650	650	650	*650*	...
46	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	1	4	19	210	380	440	500	500	550	550	650	900	900	900
47	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	39	92	170	240	340	450	450	550	550	550	600
48	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	5	12	170	300	400	400	400	400	400	400	500	650	650
49	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	5	75	155	157	165	250	290	290	400	400	400	450
50	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	1	5	60	120	240	240	300	400	500	500	500	500	650
51	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	85	150	170	210	250	350	350	450	600	600	750
52	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	1	2	6	7	27	47	120	175	200	240	240
53	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	26	44	64	104	160	220	300	300	350	580	580
54	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	4	5	8	10	43	100	170	260	260	260	260
55	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	2	13	58	85	110	120	140
56	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	1	6	9	30	45	64	64	75
57	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	3	8	26	34	40	50	70
58	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0	0	0	0	3	3
59	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0	0	0	0	0
60	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0	0	1	1
61	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0	0	0
62	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0	0
63	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0	0
64	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	0	0
65	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0
66	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0

During the first half of the season infection did not take place in the heart and young leaves, but later, as in August, during a heavy attack by the fungus they became infected soon after they were large enough to be tagged. This might be explained by the fact that since many of the

leaves were killed by the fungus, the plants were forced to produce more new leaves in an effort to keep up their normal activities. Under such conditions the new leaves formed, appeared to mature earlier than usual, and never became as large as normal. Thus, they became susceptible to infection by *Cercospora beticola* quite early in their development, and often became infected while comparatively small.

This difference in the susceptibility of the different leaves is shown in a general way in Table XI by the diagonal grouping of the three types of leaves—namely, the very young, the mature, and the old. The upper diagonal indicates either no increase in spots on the old leaves, or a slight increase on those which were still somewhat active. The lower diagonal indicates the very young leaves on which there occurred few or no spots, while the middle section represents the mature, active leaves of the plant on which the greatest increase in infections took place. A great increase in the number of infections developed on either the same leaf (reading to the right) or on the entire plant (reading diagonally) as the season advanced.

The mature leaves therefore show the greatest susceptibility to leafspot infection and possess the characters which allow the freest penetration of the host tissue by the fungus. Such leaves, as previously shown, have on the average a stomatal count on the upper surface of approximately 100 per square millimeter with a stomatal pore length of 28μ and exhibit the greatest stomatal movement. Thus, the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, for they both occur on the leaves of the same degree of maturity.

STOMATAL MOVEMENT AND GERM-TUBE PENETRATION

It may then be concluded that a favorable daily temperature (70° to 90° F.), combined with a relative humidity which does not fall below 60 at any time, together with daylight, will offer conditions under which the stomata on the mature leaves should remain open throughout the day. This condition of the host associated with favorable growth factors for the parasite would usually allow germ-tube penetration and leafspot development.

With these factors active in producing stomatal opening, detailed studies were made of germ-tube penetration from material that had been collected in the field during controlled tests. For these experiments newly formed conidia from recently developed leaf spots were sprayed on mature sugar-beet leaves about 7 p. m. After an incubation period of 11 days numerous typical leaf spots appeared. Portions of these leaves were taken 24, 36, 48, 60, and 72 hours after inoculation, killed and stained according to modifications [1] of the method given by Vaughan

[1] These modifications were suggested by Miss Pearl M. Smith, of the Botany Department of the University of Wisconsin. After the acetic alcohol had acted for 12 to 24 hours, the material was washed for 6 to 8 hours in 95 per cent alcohol, stained in Pianeze's stain overnight, and destained with acid alcohol until the leaf tissue became a clear red, or even pink in places. The material was washed in 95 per cent alcohol until the acid was removed and mounts made in Euparal. Balsam, as a mounting medium in these studies, was not found to give a good differentiation between the stomata and the penetrating fungous mycelium.

(11). An examination of several hundred slides prepared at different times by this method from inoculated leaves has shown that conidia may germinate, produce long germinating tubes and yet not penetrate closed stomata (fig. 6). On the other hand, wherever penetration was found to occur, the stomata were open, and although it has long been known that this organism gains an entrance through the stomata, this point has never been mentioned. Thümen (9, p. 50–54) seems to have been

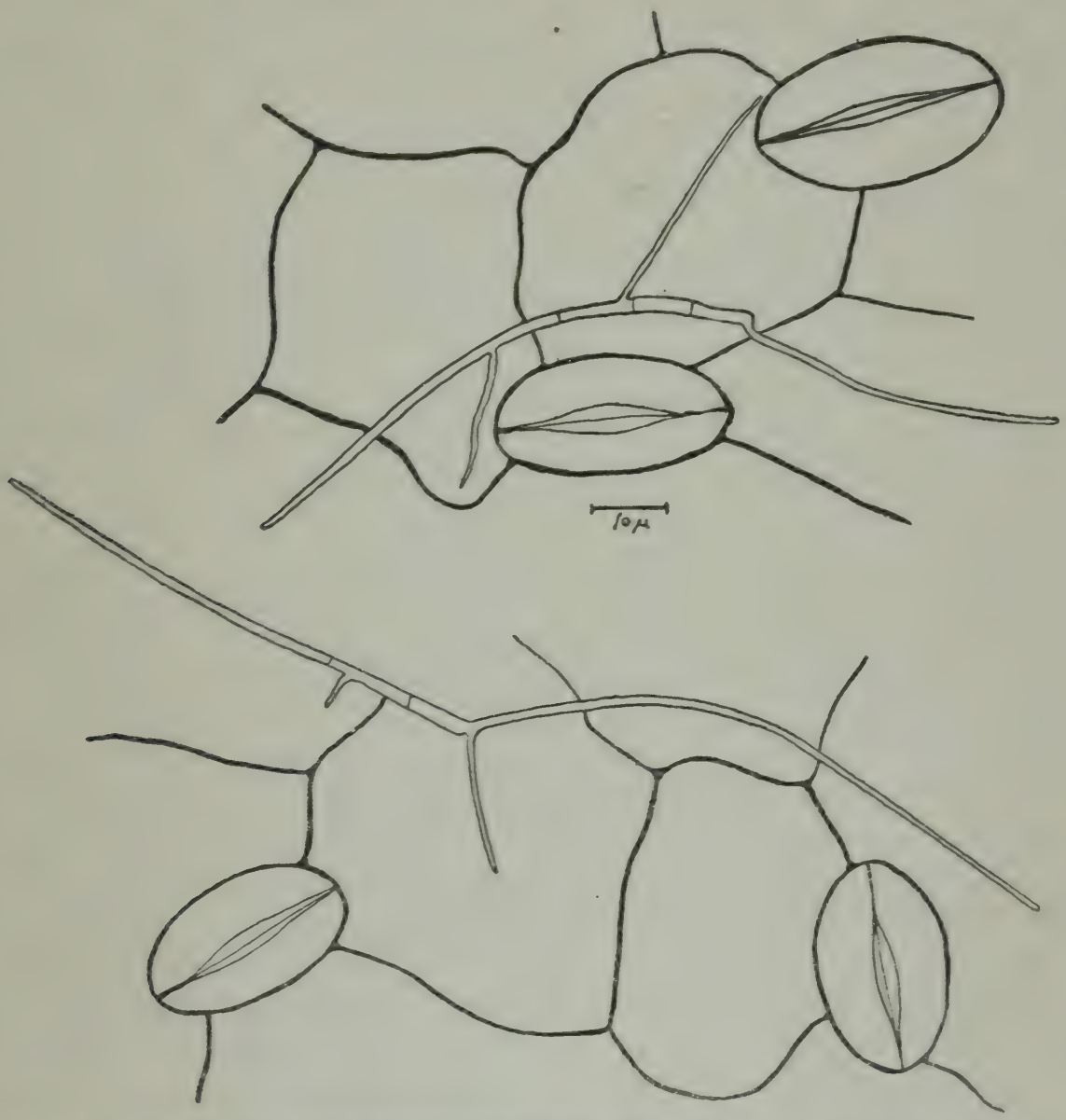

FIG. 6.—*Cercospora beticola:* Conidia germinating on a sugar-beet leaf, but germ tubes not entering or being greatly attracted by closed stomata.

the first to state that a spore which is carried by some means to a green and yet not too old, and thereby hardened, beet leaf, is able to germinate in the shortest time, penetrate into a stoma, and form a number of hyphæ. Frank (3) also agrees with this observation, adding that it is characteristic that the tufts of conidiophores grow out of the stomata. However, no mention seems to be made of the stomatal movement necessary for host penetration.

As soon as penetration of the stoma was gained by the germ tube, a marked change was noted to take place in the character of the fungous growth produced, as indicated by different staining qualities. The conidium and the slender germ tube external to the spore opening stained lightly, while the cells in the pore opening or beneath the stoma stained much more deeply and were comparatively large and round (Pl. LXXXI, *A*, *B*, *F*). It was only rarely observed that penetration into two different stomata took place by germ tubes from one conidium (Pl. LXXXI, *B*, b). In the case observed, the two stomata were near each other and slight germ-tube growth was sufficient for the penetration of both. As a rule, however, only one germinating tube from a conidium has been found to penetrate the host tissue, although it is known that, if this tube does not penetrate before its desiccation takes place, another cell of the conidium may germinate later before the entire conidium loses its viability and penetration might again be possible. At times the pore wall of a guard cell may be penetrated and the growth gradually spread to the adjoining epidermal cells (Pl. LXXXI, *F*, *c*). Normally, however, the germ tubes grow through the pore opening, probably receiving some stimulus from the guard cells and form round, heavily staining mycelial cells which pile up directly in the air chamber below the pore opening. The fungus then grows toward the parenchyma cells (Pl. LXXXI, *C*, *d*) and flatten out against their walls, probably for nutritive purposes. At times, without further development within the host, the fungus grows back out through the stoma and produces conidiophores (Pl. LXXXI, *D*, *e*). In such a case new conidia might be produced before an extensive area of the host tissue had been killed. Usually, however, the fungus grows farther into the host before conidia are formed. It probably is true, as first suggested by Uzel (10), that the fungus causes asphyxiation and consequent collapse of the parenchyma cells, since only a slight intercellular growth of the fungus occurs. An attempt by the host cells to isolate the invading organism is seen in the massing of heavily staining substances (Pl. LXXXI *E*, *f*) in the parenchyma cells which adjoin the air chamber. Under certain conditions this isolation probably is accomplished and the host cells then remain turgid and normal. Where this can not be done, the cells surrounding the fungous mycelium collapse (Pl. LXXXI, *G*), the mycelium gradually produces tufts of conidiophores, and the characteristic leafspot is formed. The host under normal growth conditions is able to isolate this infected area, though as a result of severe, abundant infections, entire leaves may be covered with the conidiophore tufts of the fungus.

It then appears that there is no attractive force existing between the closed stomata and the conidial germ tubes of the fungus, and also that the latter do not possess enzymic power to directly penetrate the epidermal cells. However, with open stomata germ-tube penetration may occur, even though some length must be attained before the tube can

reach the pore opening. The reaction upon penetration induces a great change in the type of fungous growth, the fungous cells becoming large and round. It is to be concluded that since growth continues immediately in the air chamber below the stomata, the stomatal function of gaseous interchange is needed for the development of the mycelium in the host, as well as a force for initial penetration. It seems evident, therefore, that since germ-tube penetration may occur only when the stomata are open, and since stomatal movement is directly related to daylight hours, infection takes place only at this time.

SUMMARY

The study of the relation of stomatal movement to infection of the sugar-beet plant by *Cercospora beticola* Sacc. has revealed that certain morphological and environmental factors influence stomatal activity, and, in turn, the latter, together with a favorable growth of the fungus, influences infection.

Leaf maturity, light, temperature, and relative humidity are factors concerned with stomatal movement.

Leaf maturity may be determined by two characters which for any given stage have been found to remain uniform—i. e., the number of stomata present per square millimeter of leaf surface, and the length of the stomatal pore. These characters, taken together, give a good indication of leaf maturity, regardless of leaf size or position on the plant Leaf maturity has a direct relation to stomatal activity in that movement is greater on mature than on young leaves, while on old leaves only very slight movement has been observed.

Light is probably one of the fundamental environmental factors that influence stomatal movement, and while direct sunlight may have an accelerating action, it is not essential for stomatal opening, since stomata may open widely in the shade.

Good stomatal opening has been obtained at temperatures ranging from 70° to 90° F. With these optimum temperatures active, relative humidity, with its associated causes and their effects, greatly influences stomatal movement. A high humidity favors stomatal opening, while a low humidity is associated with closure of the stomata. If the humidity remains above 60 through the day hours, the stomata will probably remain well open; but if it falls much below 50, stomatal closure will probably result.

Some of the factors influencing infection of beet leaves by *C. beticola* are rapidity of germ-tube growth, maturity of the leaves, and stomatal movement.

Fresh viable conidia of *C. beticola* germinate equally well and grow rapidly in distilled water, soil decoction, irrigation water, and bean decoction, in either darkness or diffused light at 24° C.

Infection, both artificial and natural, occurs best on mature leaves, and this is associated with the movement of the stomata.

Penetration of the leaf by the conidial germ tubes of *C. beticola* has been observed to occur only through *open* stomata, and consequently infection probably takes place during the day hours. An isolation of the invading organism is attempted by the leaf cells as soon as penetration occurs, but when this is not successful, the fungus by further growth produces a well-defined leafspot.

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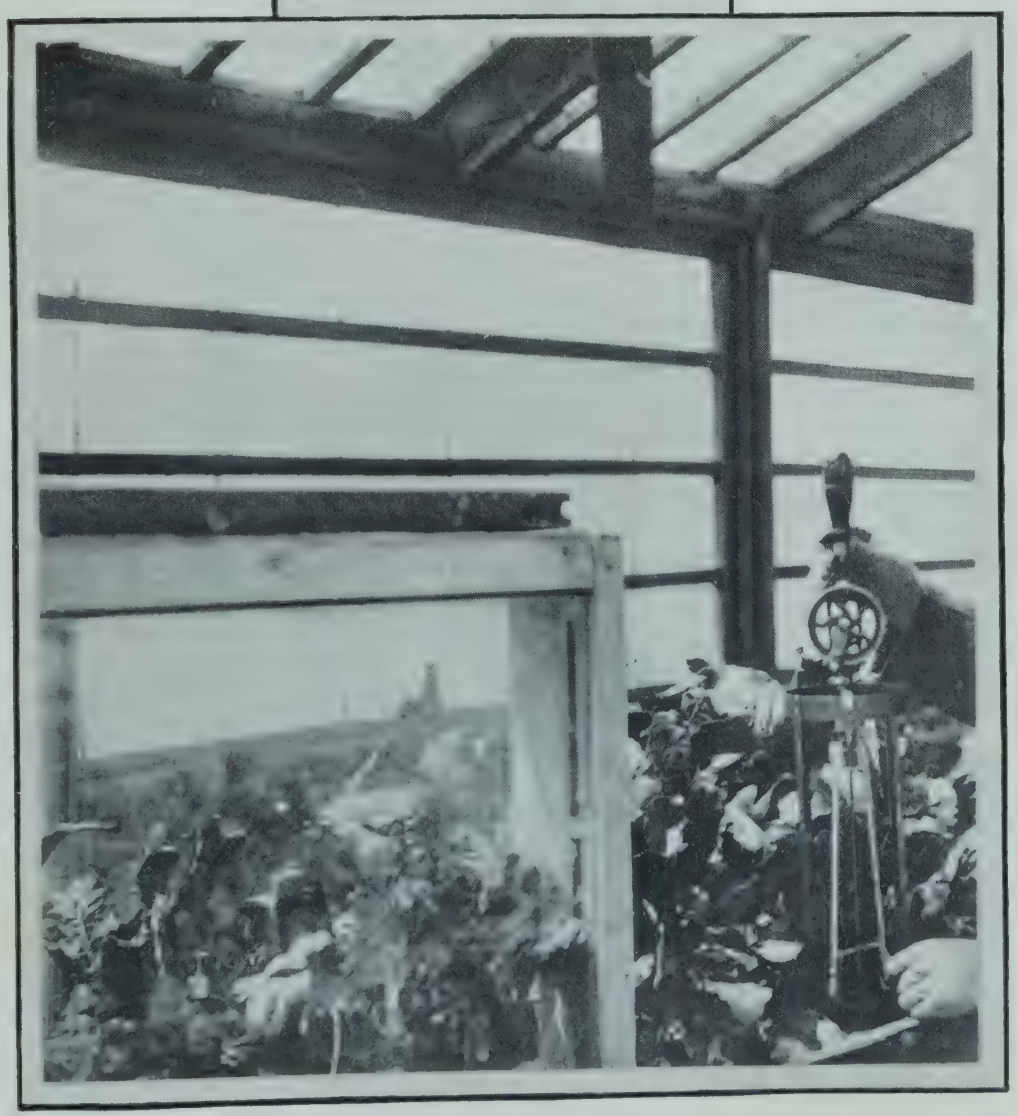

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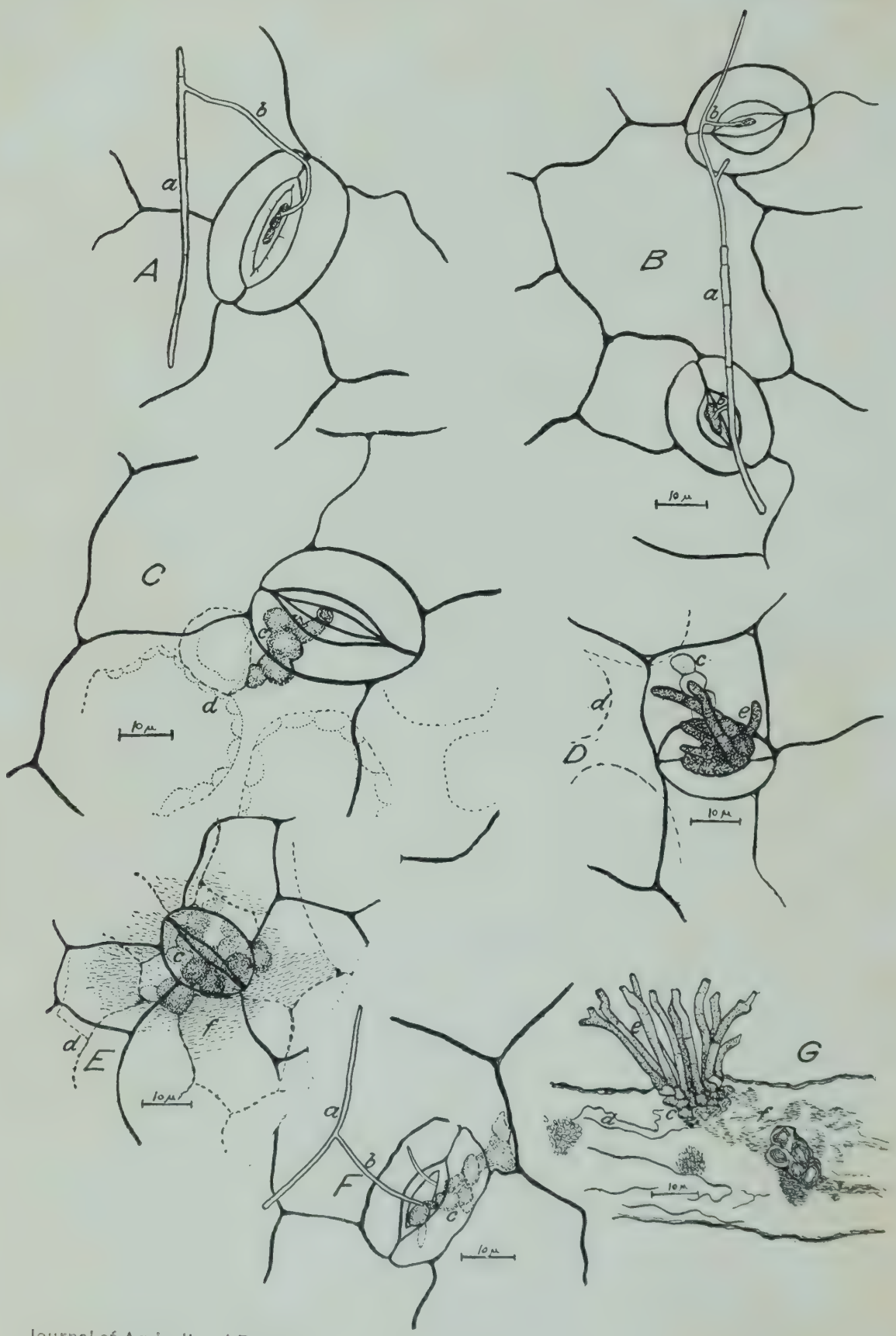
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A METHOD OF CORRECTING FOR SOIL HETEROGENEITY IN VARIETY TESTS[1]

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Men with practical experience in conducting variety tests and fertilizer experiments are free to admit that in many cases the results of ordinary field trials are of little or no value. The reason for this lies in the large number of factors which are beyond the control of the experimenter. In many instances variation in any one of these uncontrollable factors may influence the final results to a greater extent than the one controlled variable for which the experiment was undertaken.

On the other hand, field trials and variety tests play an important part in agricultural investigations. Such tests are an indispensable adjunct to plant-breeding work. The final test of new varieties or new strains must be made under field conditions. It is therefore of the greatest importance that methods should be devised which will in some measure at least take account of these uncontrollable factors.

No one of these factors is of more importance than the variation in the soil in different plots. It is practically impossible to secure for such field trials a tract of land that is absolutely uniform. The literature of variety tests abounds in illustrations of this fact.

In 1897 Larsen (8),[2] on the basis of results with timothy, reached the conclusion that more exact results were obtained where a given area was divided into a large number of plots than when it was divided into a few larger ones.

Holtsmark and Larsen (7) extended this idea and supplied additional evidence. Hall (1) in 1909 and Mercer and Hall (9) and Hall and Russell (2) in 1911 laid great emphasis upon soil heterogeneity in field tests. Among other things they did much to determine the most suitable sizes for experimental plots.

Montgomery (10, 11) has produced evidence showing that systematic repetition of plots over a given area reduces the variability in proportion to the number of repetitions; further, that while increase in the size of a plot decreases the variability up to a certain limit, a further increase in size is not attended by a corresponding decrease in variability.

As a result of these several investigations, it has become evident that much more reliable results are obtained by using several systematically repeated small plots than by using a single large one. This method is rapidly coming into more general use in field tests of all kinds. Never-

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 93.
[2] Reference is made by number to "Literature cited," p. 1050.

theless, where for various reasons it is impossible to make a large number (10 to 20) of repetitions, the factor of soil heterogeneity still enters into the average yield. One or two exceptionally high or exceptionally low yields will unduly influence the average where the number of repetitions is only four or five.

In a series of papers Harris (3, 4, 5, 6) has called attention to various phases of the experimental error in field tests. In his most recent paper on this subject Harris (6) has proposed a method of measuring the heterogeneity of the soil of a field. The principle employed by Harris is stated thus (432–433):

> If the irregularities in the experimental field are so large as to influence the yield of areas larger than single plots, they will tend to bring about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

This tendency to grouping of the high- and low-yielding plots is evident in most field experiments. It is clearly shown in the diagrams published by Montgomery (10).

The measure which Harris proposes for this heterogeneity (or homogeneity) of a field is the correlation between the yield of the ultimate small plots and the yield of various groups of contiguous plots. The more nearly this correlation approaches zero the more homogeneous the field. The more differentiated a given field is in regard to good and poor soil, the greater will be the value of the correlation coefficient.

This method of measuring the heterogeneity of a field is dependent somewhat upon the size of the ultimate plots and also upon the method of grouping. It does, however, mark a distinct advance in our method of dealing with small plot experiments.

While Harris's method provides a *measure* of the substratum heterogeneity in a given field, it does not provide any means of obtaining a corrective term for individual plots. While in field experiments it is of importance to know the amount of heterogeneity in the field as a whole, it is usually of much more importance to obtain some correction to apply to individual plots which will in some measure even up the differences in soil conditions.

The present paper is the result of an attempt to obtain such a corrective term. It is realized that the method proposed is far from ideal. It is believed, however, that it marks a step in this direction, and it is hoped that it may lead to further study of this important question.

The usual method of taking account of soil heterogeneity is the use of check plots. However, in very many cases this method has been far from satisfactory. It is not at all difficult to find examples in the literature of variety tests in which the amount of variation in the check plots is nearly or quite as great as the variation in the other varieties.[1] If check

[1] Davenport, Eugene, and Fraser, W. J. Experiments with wheat, 1888–1895. Experiments with oats, 1888–1895. Ill. Agr. Exp. Sta. Bul. 41, p. 147–160. 1896.
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plots are repeated at sufficiently frequent intervals, they will undoubtedly be a great aid in determining the correction for soil differences. However, where field tests of this kind are carried out on even a moderate scale, the use of check plots adds very materially to the labor and expense of the experiment. For example, in 1914 we grew 150 one-fortieth acre plots. From a study of the field it seems clear that any adequate system of checks would have required 1 check plot to every 5, or about 30 additional plots. The labor involved in handling these would have been considerable; and judging from the literature on the subject, the value of the results might still be very doubtful.

For several years this Station has been carrying on variety tests of oats. The object of these tests is to obtain some measure of the productiveness of new strains or varieties produced in the plant-breeding work. These new strains are always tested along with a number of standard commercial varieties. The method adopted in this work (13) is to grow four systematically repeated plots of each variety. The size of each plot is 33 feet square, or one-fortieth of an acre. The four plots thus make a total of one-tenth of an acre devoted to each variety. These plots have always been grown on a more or less rectangular piece of ground. (See fig. 4.) The fields for these tests have been chosen for their apparent uniformity. However, the resulting yields have always indicated that certain portions of the field were much better or worse in respect to soil fertility than the average of the field as a whole. In certain cases two or more of the four plots of a variety come to lie, say, in certain of these more fertile spots. This tends to produce an unduly high average for that variety.

In order to obtain a correcting value for these different soil conditions, it occurred to us to determine first the probable yield of each plot by the contingency method. This may be done as follows: Take a theoretical field divided into plots as in figure 1. Let a, b, c. . . . l represent the observed yields of the respective plots, of which the mean yield is p. Then, assuming all plots to be planted with the same variety and conditions other than the soil to be uniform, we can obtain the most probable yield of, say, plot a by multiplying the sum ac by the sum aj and dividing by the total al. Proceeding in this way for each plot, we can obtain a calculated yield a', b', c'. . . . l' for each plot. The mean of these calculated yields will be the same as the mean of the observed yield—viz, p.

It is clear that these so-called calculated yields correspond to what Pearson (12) in his work on contingency has designated by ν_{uv}, or the value for each square on the hypothesis of independent probability. The difference between the observed and calculated yields would then correspond to what Pearson calls a subcontingency.

The "calculated" yields obtained by this contingency method represent the most probable yields of the respective plots based on the distribution of the observed yields. This method of estimating the probable

yield takes into account the soil differences in both directions across the field. To a certain extent it is dependent upon the assumption that the soil changes in a uniform manner from one side of the field to the other. Harris (6) has pointed out that this is not always the case, but that the diagrams of experimental fields indicate that differences in soil are more likely to occur as a spotting of the field. However, a closer study of the observed yields in many experimental fields indicates that there is a tendency for areas of good soil (high yield) to grade off through areas of medium soil to regions of poor soil. Ordinarily, the changes from one extreme to the other are not abrupt (see fig. 3, 4). The diagrams published by Montgomery (10) indicate this to some extent, although such diagrams do not show the graded changes as well as a study of the actual yields of contiguous plots.

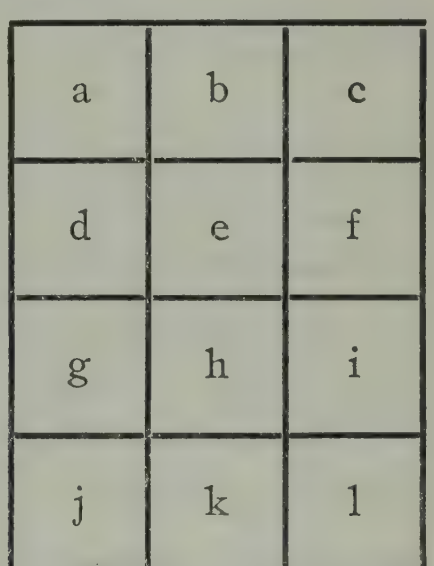

FIG. 1.—Diagram illustrating the method of obtaining the "calculated" yield. (For explanation, see text.)

Further, if the distribution of the high and low "calculated" yields in figures 2 and 3 are compared with the high and low observed yields, it will be seen that the former show approximately the same "spotting" as the latter. This method does tend to lessen the variability and to smooth the results. While it is not ideal and does not obviate all the difficulties, it seems possible that this method may prove useful in estimating soil differences.

For cases like Montgomery's wheat experiment (10) or Mercer and Hall's field trials (9), where there are a number of plots all planted with the same variety, the contingency calculated yields may be used directly. For such experiments these calculated yields represent a smoothing of the original observations. In the case of field trials or variety tests, where different plots have different treatments or are planted with different varieties, such a smoothing tends to mask the actual differences between the plots. In such cases a further procedure is necessary.

In the case of a variety test the yield calculated by this contingency method may be regarded as the most probable yield of any given plot if we suppose the whole field had been planted with a single variety whose average yield was the same as the observed average of all the plots. The deviation of the calculated yield of a given plot from the mean of the field may be taken as a measure of the influence of the soil of that plot as compared with the whole field. Thus, if the calculated yield of a given plot is 10 bushels above the average of the field, it may be taken to mean that the soil on this plot is capable of producing 10 bushels more grain than the soil on the field as a whole.

This figure may be used to correct the observed yield of the corresponding plot. Thus, if the observed yield in a given plot is 80 bushels and the calculated yield is 5 bushels above the average of all the plots, then to make the yield of this plot comparable with the average of the field it would be necessary to reduce the observed yield by 5 bushels. Thus, we may obtain for this plot a "corrected" yield of 75 bushels.

Likewise, where the calculated yield is below the average, it is necessary to add a corresponding amount to the observed yield in order to take account of the deficiency in the soil of that plot.

Expressed in a formula, we may let O equal the observed yield and D the deviation of the calculated yield from the mean of the field. Then the

$$\text{"corrected" yield} = O - D$$

In fields where there are comparatively small differences between the yield of individual plots the direct method of correcting the yield as given above may be used. The corrected yields given in figures 2 and 3 were obtained by this direct method.

In the case of variety tests or experiments where there are likely to be marked differences between individual plots, it will be better to make corrections on a relative rather than an absolute basis. To do this, the deviation of the calculated yield from the mean of the field is determined as before. Next the percentage which each deviation is of the mean is determined. Then this percentage of the observed yield is added to, or subtracted from, the observed yield to obtain the corrected yield. An example will make this clear. Suppose the mean yield of the plots in a field is 70 bushels. The observed yield on a given plot is 80 bushels and the calculated yield of this plot is 77 bushels. Thus, the deviation of the calculated yield from the mean is +7 bushels, which is 10 per cent of the mean (70 bushels). The corrected yield will then be 10 per cent less than the observed; or 10 per cent of 80 equals 8 bushels. The resulting corrected yield will be 72 bushels. By the absolute method the corrected yield would have been 73 bushels. The corrected yields given in figure 4 and Table I have been obtained by this method.

It is next of importance to see whether this "corrected" yield has really obviated any of the difficulties. To test this, use may be made of the criterion of soil homogeneity proposed by Harris (6). This can best be tested upon such data as those furnished by the experimental fields of Montgomery (11) or Mercer and Hall (9).

Figure 2 is a diagram taken from Montgomery (11). It represents a field of Turkey wheat grown in 1908–9. This field was divided into 224 blocks (each 5.5 feet square), as indicated. The grain from each block

671 692	657 697	703 701	755 714	760 703	686 665	592 590	739 712	732 688	710 648	753 646	680 654	680 559	677 684	795 762	723 683
658 672	713 746	613 604	632 583	667 603	645 626	660 652	768 734	786 734	768 698	666 550	843 809	795 767	763 763	716 675	741 693
657 644	671 678	623 587	715 637	543 449	613 557	640 604	798 735	759 678	764 664	995 847	793 731	936 880	755 728	792 722	838 761
642 644	680 701	654 632	673 610	760 682	709 668	682 661	724 677	774 709	860 776	787 657	725 678	664 623	851 838	690 636	770 681
735 744	580 608	620 605	675 620	765 695	742 708	772 758	698 658	652 594	661 584	768 646	777 738	745 711	768 762	851 804	719 665
575 613	598 654	705 720	642 619	704 695	643 640	650 666	572 563	752 726	740 696	863 776	680 672	722 719	723 747	703 688	756 734
727 772	633 696	615 638	685 670	662 632	639 644	657 680	608 607	620 602	624 588	745 666	764 764	703 708	752 784	788 781	682 668
572 664	373 500	560 622	645 682	692 715	644 699	632 705	574 624	606 640	648 666	806 784	791 841	629 684	650 730	679 723	588 626
580 641	425 504	732 771	730 732	706 694	732 754	736 776	655 672	673 673	793 776	765 705	576 593	609 631	568 616	728 738	620 623
588 649	526 605	596 636	777 780	776 765	779 801	721 762	728 745	604 673	742 725	665 606	621 638	611 633	623 671	646 657	617 621
617 682	683 765	726 770	835 842	668 661	664 690	691 735	770 801	775 779	685 672	723 668	583 604	580 606	395 447	511 526	653 661
602 710	662 786	640 720	700 753	650 690	655 727	563 652	600 677	730 781	690 725	713 709	530 597	568 640	410 506	478 539	636 690
665 726	736 815	630 670	598 601	895 883	592 614	593 633	659 686	718 718	705 688	667 608	585 602	560 582	655 704	633 644	733 736
609 682	706 797	790 842	678 753	695 697	715 750	622 675	658 697	597 610	632 628	713 668	585 615	657 692	495 556	618 641	652 668

FIG. 2.—Diagram showing the observed and corrected yield (in grams) of grain on each of Montgomery's wheat plots in 1908–9. The upper figure in each plot is the observed yield and the lower the corrected.

was threshed and weighed separately. The upper figure in each square is the observed yield of grain in grams. The lower figure is the corrected yield obtained by the method outlined above. The mean yield of these plots is taken as 681 gm.

Figure 3 represents the combination plots obtained by grouping the plots in figure 2 in groups of four—i. e., a two- by two- fold grouping. In this figure the upper number in each plot is the observed yield, the lower number the corrected yield, while the middle number is the "calculated" yield. This latter is inserted to illustrate the method of obtaining the corrected yield. The mean yield of these grouped plots is taken as 2,723 gm.

Now, if we calculate the correlation between the observed yield of the ultimate plots and the observed yield of the combination plots it is found that

$$r = +0.358 \pm 0.039$$

This shows a fairly large coefficient of correlation, indicating a relatively large heterogeneity in the soil of this field.

If we calculate the correlation between the corrected yields of the ultimate plots and the corrected yields of the combination plots it is found that

$$r = +0.111 \pm 0.045$$

This coefficient is less than three times its probable error and is hardly to be regarded as significantly greater than 0. In any case it indicates that this method of correcting the yields has practically, if not quite, eliminated the influence of differences in soil of different plots.

2,699	2,703	2,758	2,759	2,996	2,942	2,915	2,975
2,616	2,826	2,894	2,798	2,953	3,007	2,766	2,887
2,806	2,600	2,587	2,684	2,766	2,658	2,872	2,811
2,650	2,665	2,625	2,844	3,157	3,300	3,206	3,090
2,707	2,924	2,994	2,895	3,056	3,112	2,862	2,987
2,666	2,464	2,354	2,672	2,824	2,911	3,067	2,826
2,488	2,642	2,854	2,692	2,805	3,088	2,958	3,029
2,594	2,802	2,869	2,774	2,928	2,982	2,743	2,863
2,617	2,563	2,708	2,641	2,600	2.829	2,938	2,889
2,305	2,505	2,637	2,471	2,498	3,106	2,734	2,737
2,414	2,608	2,670	2,583	2,725	2,776	2,553	2,664
2,614	2,620	2,690	2,611	2,496	3,053	2,904	2,796
2,119	2,835	2,993	2,840	2,812	2,627	2,411	2,611
2,444	2,640	2,703	2,614	2,759	2,809	2,584	2,697
2,398	2,928	3,013	2,949	2,776	2,541	2,540	2,637
2,564	2,901	2,637	2,624	2,880	2.549	1,953	2,278
2,345	2,533	2,593	2,508	2,647	2,694	2,479	2,587
2,942	3,091	2,767	2,839	2,956	2,578	2,197	2,414
2,716	2,696	2,897	2,532	2,652	2,550	2,367	2,636
2,421	2,615	2,677	2,589	2,732	2,782	2,559	2,671
3,018	2,804	2,943	2,666	2,643	2,491	2,531	2,688

FIG. 3.—Diagram showing the observed, corrected, and calculated yield (in grams) of Montgomery's wheat plots in groups of four, taken from figure 2.

Similar coefficients have been calculated for other fields with corresponding results.

It will next be of interest to test this method in the case of an actual variety test. This has been done in the case of all of our own variety test fields. The results will be published in another place in connection with a discussion of some pure-line oat varieties. In order to furnish an example of the use of this method in a variety test, the results of our 1915 test of oat varieties are given below.

Figure 4 represents a diagram of the 1915 plots of oats at the Highmoor Farm (Monmouth, Me.). In the upper left-hand corner of each square is the plot number as it occurs in our records. Immediately below this is the name of the variety. In the case of the pure-line varieties these are

indicated by our own record number—for example, as Maine 340, Maine 357, etc. The upper of the two remaining numbers in each square is the observed yield and the lower number is the corrected yield. All yields are given in bushels per acre.

905 Irish Victor 75.93 78.34	904 Maine 336 73.75 77.11	903 Siberian 72.12 72.02	902 Maine 230 75.25 76.42	901 Banner 73.75 74.85	900 Maine 351 77.75 79.34	899 Swedish Select 68.75 75.16	898 Maine 357 85.94 79.03
913 Maine 247 84.37 85.20	912 Senator 50.87 52.08	911 Maine 281 83.37 81.36	910 Maine 891 82.75 82.19	909 Minn. 26 90.93 90.27	908 Maine 340 82.75 82.60	907 Kherson 53.12 56.99	906 Maine 337 88.75 79.43
921 Maine 286 85.62 85.79	920 Early Pearl 85.00 86.38	919 Maine 346 69.38 67.14	918 Imported Scotch 70.31 69.26	917 Maine 307 76.87 75.70	916 Gold Rain 83.37 82.55	915 Maine 355 73.75 78.57	914 Prosperity 77.50 68.68
929 Maine 336 72.19 72.70	928 Siberian 80.62 82.33	927 Maine 230 74.62 72.61	926 Banner 90.31 89.45	925 Maine 351 78.37 77.59	924 Swedish Select 67.50 67.19	923 Maine 357 73.75 78.93	922 Maine 918 81.25 72.47
937 Senator 61.25 62.35	936 Maine 281 68.75 70.96	935 Maine 978 82.50 81.21	934 Minn. 26 80.25 80.38	933 Maine 340 84.00 84.10	932 Kherson 66.25 66.69	931 Maine 337 81.25 87.79	930 Irish Victor 87.50 79.12
945 Early Pearl 96.50 87.07	944 Maine 346 82.50 75.73	943 Imported Scotch 68.75 59.45	942 Maine 307 82.12 72.59	941 Gold Rain 86.87 76.74	940 Maine 355 93.12 82.83	939 Prosperity 83.37 81.05	938 Maine 247 90.62 70.24
953 Siberian 75.25 74.80	952 Maine 230 76.25 76.90	951 Banner 83.45 80.08	950 Maine 351 73.12 71.45	949 Swedish Select 65.25 63.74	948 Maine 357 82.12 80.67	947 Maine 982 85.62 90.59	946 Maine 286 85.62 75.13
961 Maine 281 81.25 82.94	960 Maine 1053 78.75 81.49	959 Minn. 26 81.25 80.23	958 Maine 340 75.31 75.64	957 Kherson 73.75 74.05	956 Maine 337 73.75 74.45	955 Irish Victor 61.87 67.01	954 Maine 336 84.06 76.27
969 Maine 346 76.87 83.56	968 Imported Scotch 64.65 71.12	967 Maine 307 77.50 81.83	966 Gold Rain 71.56 71.10	965 Maine 355 78.13 83.71	964 Prosperity 70.00 75.36	963 Maine 247 70.00 80.17	962 Senator 60.00 58.88
977 Maine 230 85.62 81.40	976 Banner 85.62 82.68	975 Maine 351 80.62 73.76	974 Swedish Select 71.87 67.08	973 Maine 357 76.87 71.70	972 Maine 1054 84.37 79.19	971 Maine 286 77.81 79.18	970 Early Pearl 90.87 75.35
985 Maine 1064 56.56 60.19	984 Minn. 26 74.37 80.13	983 Maine 340 89.00 91.86	982 Kherson 66.25 69.46	981 Maine 337 71.56 74.99	980 Irish Victor 76.25 80.31	979 Maine 336 65.94 74.10	978 Siberian 83.12 79.43
993 Imported Scotch 55.00 59.38	992 Maine 307 71.25 77.86	991 Gold Rain 77.50 81.23	990 Maine 355 83.75 89.13	989 Prosperity 67.12 71.41	988 Maine 247 75.62 80.83	987 Senator 51.25 58.34	986 Maine 281 91.87 89.39
76.74 78.38	76.74 79.45	76.74 75.81	76.74 77.13	76.74 77.09	996 Maine 286 61.88 62.50	995 Early Pearl 74.06 80.27	994 Maine 346 90.00 81.72

FIG. 4.—Diagram showing the yield of oats (in bushels per acre) on the 1915 variety-test field at Highmoor Farm (Monmouth, Me.) Each square represents a one-fortieth acre plot. (For description see text.)

In this field there were tested 11 commercial varieties and 12 pure-line varieties in quadruplicate one-fortieth acre plots. In addition, seven other pure lines were tested in single plots. It will be noted that in the lower row of the figures there are five plots not planted. In order to use this method of correction, it is necessary to assign values to these plots. The best method of doing this is to assign as the observed yield of each such plot the mean yield of the field. This method does not bias the results in either direction.

Table I shows the average yield, both observed and corrected, for the four plots of each commercial variety and for the 12 pure-line varieties. These corrected yields have been obtained by the percentage method described above.

TABLE I.—*Variation constants for the observed and corrected average yields of commercial and pure-line varieties of oats tested in 1915*

COMMERCIAL VARIETIES

Variety.	Observed yield (bushels per acre).	Standard deviation.	Coefficient of variation.	Corrected yield (bushels per acre).	Standard deviation.	Coefficient of variation.
Minnesota No. 26	81.70±2.00	5.94±1.41	7.27±1.74	82.75±1.46	4.34±1.03	5.24±1.25
Early Pearl	86.61±2.80	8.31±1.98	9.59±2.31	82.27±1.61	4.79±1.15	5.82±1.39
Banner	83.28±2.03	6.04±1.44	7.25±1.73	81.77±1.77	5.26±1.25	6.43±1.53
Gold Rain	79.83±1.84	5.48±1.30	6.86±1.64	77.90±1.50	4.48±1.06	5.75±1.37
Siberian	77.78±1.45	4.33±1.03	5.57±1.33	77.14±1.34	4.00± .95	5.18±1.23
Irish Victor	75.39±3.06	9.09±2.16	12.06±2.91	76.19±1.80	5.35±1.27	7.02±1.68
Prosperity	74.50±2.14	6.37±1.51	8.55±2.05	74.13±1.56	4.65±1.10	6.27±1.50
Swedish Select	68.34± .80	2.39± .56	3.50± .83	68.29±1.41	4.20±1.00	6.15±1.47
Kherson	64.68±2.50	7.43±1.77	11.46±2.76	66.79±2.10	6.25±1.49	9.36±2.25
Imported Scotch	64.68± .63	1.88± .44	2.91± .69	64.80±1.83	5.43±1.29	8.38±2.01
Senator	55.84±1.62	4.81±1.14	8.61±2.06	57.92±1.11	3.32± .79	5.73±1.37
Average	73.89	5.64	7.63	73.63	4.73	6.48

PURE-LINE VARIETIES

No. 340	82.77±1.65	4.90±1.16	5.92±1.41	83.55±1.94	5.76±1.37	6.89±1.65
No. 355	82.19±2.44	7.24±1.72	8.81±2.11	83.55±1.26	3.76± .89	4.50±1.07
No. 281	81.31±2.78	8.27±1.97	10.17±2.45	81.16±2.22	6.61±1.57	8.14±1.94
No. 337	78.83±2.27	6.76±1.61	8.58±2.06	79.17±1.79	5.34±1.27	6.74±1.61
No. 247	80.15±2.66	7.92±1.88	9.88±2.16	79.11±1.84	5.47±1.30	6.91±1.65
No. 357	79.67±1.58	4.70±1.12	5.90±1.41	77.58±1.16	3.47± .82	4.47±1.06
No. 230	77.94±1.50	4.47±1.06	5.73±1.37	77.58±1.05	3.12± .74	4.02± .95
No. 346	79.69±2.54	7.56±1.80	9.49±2.28	77.04±2.16	6.41±1.52	8.32±1.99
No. 307	76.94±1.30	3.86± .92	5.02±1.20	77.00±1.13	3.36± .80	4.36±1.04
No. 286	77.73±3.26	9.69±2.31	12.47±3.01	75.65±2.86	8.49±2.02	11.22±2.70
No. 351	77.47± .91	2.73± .65	3.52± .84	75.54±1.04	3.10± .73	4.10± .97
No. 336	73.99±2.19	6.51±1,55	8.79±2.11	75.05± .58	1.74± .41	2.32± .55
Average	79.06	6.22	7.86	78.50	4.72	6.00

From figure 4 it is seen that in many plots the corrected yield varies quite widely from the observed. However, Table I shows that when the four plots of each variety are averaged there are in most cases comparatively slight differences between the two. This point is a strong argument for the efficiency of four systematically repeated plots in reducing the experimental error. There are, however, a few cases in the table

where the corrected average yield is markedly different from the observed. An instance of this is seen in the Early Pearl variety (Table I). The observed average yield of this variety (86.6 bushels) was the highest obtained in 1915. The difference between the yield of this and the Minnesota No. 26 was nearly 5 bushels. The corrected average yield of these two varieties is practically the same, differing only in a fraction of a bushel. By referring back to figure 4 it is found that the high average yield of the Early Pearl was largely due to the influence of two plots, Nos. 945 and 970. These two plots happened to lie in exceptionally good soil. Their observed yields of 96.5 and 90.9 bushels per acre were reduced to the corrected yields of 87 and 75.4 bushels, respectively.

As is to be expected, the corrected average yields show in nearly all cases a much lower variability. This is true of both the absolute and relative variability. In one or two instances, as the Imported Scotch (Table I), the variability is greater in the case of the corrected yield. If all the varieties (Table I) are taken, the corrected yields will show an average decrease in the coefficient of variation of about 1½ per cent.

The table shows that with systematically repeated plots the yields corrected by this method do not differ radically from the actually observed yields. Such changes in the order of yield as do occur we believe more truly express the relative value of these varieties. This statement is based on the experience of several years with these same varieties.

In using this method attention should be called to one or two points. In the first place where a field of plots is very large or where it is relatively long and narrow better results will usually be obtained by breaking it up into smaller blocks for calculation. For example, our 1914 test field was 6 plots wide and 28 plots long. More satisfactory results were obtained by breaking this up into three blocks, two of which were 9 plots long, the other 10 plots. Each block was calculated as a separate field. In doing this, care should be taken that the blocks are not so small as to be unduly affected by a possible preponderance of very good or very poor varieties.

Another point to be remembered in the practical use of this method is that it can not be used to take account of uneven seeding, ravages of birds, or other irregularities in certain plots. Corrections, if any, for these factors should be added before employing the above method.

SUMMARY

It is generally admitted that field trials, including variety tests, are often of very little value because of the large number of uncontrollable factors. Nevertheless, field trials are becoming more and more a necessity in many phases of agricultural investigation.

Within recent years a number of investigators have shown that the experimental error in such trials can be greatly reduced by the use of

systematically repeated plots. Nevertheless, if the number of repetitions is not large, certain experiments may still be unduly influenced by irregularities in the field. It would therefore be desirable if some method could be devised by which the yields of individual plots could be corrected in such a way as to take account of these irregularities.

Check plots have frequently been used for this purpose. But, aside from the extra labor and expense involved, the results from check plots have been far from satisfactory in many cases.

In the present paper a method is proposed for use in correcting for differences in the soil of different plots. The method in its present form is adapted for use only when the plots are arranged in blocks similar to those in figure 4. The method of obtaining this correction factor is as follows: In the first place the probable yield of each plot is obtained by the contingency method. This "calculated" yield represents the most probable yield of each plot on the supposition that they have all been planted with a hypothetical variety whose mean yield is the same as the observed means of the field.

This "calculated" yield may then be used as a basis for determining a correction factor. If the calculated yield of a given plot is above the mean of the field it must be taken that the soil of this plot is better than the average of the field and a corresponding amount must be deducted from the observed yield. Likewise, if the calculated yield is below the average, a proportional amount must be added to the observed yield in order to make the plots comparable.

Still more comparable results will be obtained if the correction factors are based upon the percentage of the mean rather than upon the absolute figures.

Tests of the efficiency of this method by means of the measure of soil heterogeneity proposed by Harris (6) show in all cases a very marked reduction in the amount of heterogeneity when the corrected figures are used. When tested on our own experimental plots, this method leads to results which from other evidence, we have reason to believe, more nearly represent the truth than do the uncorrected yields.

It is realized that this method is not ideal and does not obviate all the difficulties connected with soil differences in plot experiments. It is hoped that this method may prove useful in certain kinds of plot experiments and that it may lead to further study of this problem.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., MARCH 6, 1916 NO. 23

FLOW THROUGH WEIR NOTCHES WITH THIN EDGES AND FULL CONTRACTIONS[1]

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INTRODUCTION

The developments in irrigation agriculture in the arid West have caused many changes to be made in the method of delivering water to canals and to individual irrigators. The value of water increases with the increase of irrigated acreage, and the long-accepted practice of fixing the charges for water on a per-acre-per-annum basis is rapidly losing ground in favor of charges based on the volume of water delivered. When irrigators pay according to the amounts of water used, there is every incentive for them to study the water requirements of their crops and to use the least quantities they judge to be necessary. This leads to a proper economy in the use of water, permits a greater acreage to be irrigated with the available water supply, and conserves the land.

The transition from a flat rate to a rate based on the water actually used is calling for a better knowledge of the accuracy and practicability of existing measuring devices as well as the development of new devices. The weir is generally considered an accurate device for measuring water, and it doubtless is such, provided it is properly installed and the correct formula is used for determining the discharge through the notch. Weirs constitute a large proportion of the devices in use for measuring irrigation water at the present time, being principally of the rectangular notch

[1] This paper is based on experiments conducted in the hydraulic laboratory at Fort Collins, Colo., under cooperative agreement between the Office of Experiment Stations of the United States Department of Agriculture and the Colorado Agricultural Experiment Station.

or Francis type, and the Cipolletti type. Most of the weirs in use have notches with crest lengths of 4 feet or less, being such as are adapted to the delivery of water for farm units. Unfortunately, owing chiefly to the confusion of the statements contained in the literature on weirs, various standards of dimensions have been used in the construction of the weirs now in use. This lack of uniformity results in many erroneous measurements.

The basic experiments with notches having thin edges and full contractions were made by James B. Francis (5)[1] from 1848 to 1852. These have subsequently been enlarged upon by several experimenters and mathematicians. Francis made three series of experiments with rectangular-notch weirs, but the discharges were measured directly in only one series (5, p. 75–76). In each of the two other series an equal flow of water was made to pass through notches of different lengths, the crest lengths and the heads being noted. In the experiments, where the discharges were measured volumetrically, only notches of approximately 8- and 10-foot lengths were used, and the heads ranged from only 7 to 19 inches (5, p. 122–125). Most of the experiments were made with the 10-foot notch, as they were to be applied directly to the measurement of water for power purposes. Francis stated (5, p. 133) that the formula which he derived would apply to heads ranging from 6 to 24 inches, but in no case was it to be used either for heads exceeding one-third the length of the crest or for very small heads. With these limitations the formula can not be used for weirs having crest lengths of less than 1.5 feet nor for heads exceeding 2 feet. For a 1.5-foot crest the formula can be used only for a 0.5-foot head. Horton states (7) that the Francis data and formula will hold for heads from 0.5 foot to 4 feet. Francis's experiments were very carefully and conscientiously made, but were with longer notches and greater volumes of water than are usually needed in delivering water to irrigators. The Francis formula is frequently used, however, without regard to the limits which he imposed upon it, and it is not uncommon to see tables computed from it that give discharges for heads as low as 0.01 foot, with heads as high as 1 foot for a crest length of 1 foot, and for crest lengths varying from 0.5 foot to 20 feet.

The most popular weir notch has been the trapezoidal type with side slopes of one horizontal to four vertical. This type was designed and the formula deduced by the Italian engineer Cesare Cipolletti (3), with the idea of automatically eliminating the correction for end contractions necessary with the rectangular notches and thus obtaining a type of notch the discharge through which would be proportional to the length of the crest and free from error in excess of one-half of 1 per cent from any single cause. Cipolletti derived the shape of the notch by a mathematical modification of the Francis formula for the rectangular notch.

[1] Reference is made by number to "Literature cited," p. 1112–1113.

He obtained the values for the coefficient and exponent by examining Francis's experimental data and increasing Francis's coefficient value somewhat arbitrarily by 1 per cent. He also made a few experiments, but stated that his formula was subject to the limitations imposed by Francis; consequently the extension of the range of application of the formula has been an excursion into unexplored territory. The notch designed by Cipolletti was intended to measure a minimum discharge of 150 liters (5.3 cubic feet) per second and a maximum discharge of 300 liters (10.6 cubic feet) per second, thus further restricting the use of the Cipolletti formula to notches having crest lengths of not less than 3 feet nor more than 8 feet.

There is great practical need in irrigation practice for weirs with small notches and for measurements with small depths of water over the crests of the notches. It also is important to know that the discharge formulas are correct, as many other forms of measuring devices are commonly calibrated by being hitched in tandem with the weir. For these reasons it was deemed advisable to conduct a series of experiments with notches having thin edges and full contractions (1) to determine whether the Francis and Cipolletti formulas hold for notches of the sizes ordinarily used in irrigation practice and (2), in case the old formulas did not hold, to derive new formulas.

LABORATORY EQUIPMENT AND METHODS

The hydraulic laboratory at Fort Collins was built in 1912–13, under a cooperative agreement between the Office of Experiment Stations, United States Department of Agriculture, and the Colorado Agricultural Experiment Station, and is designed for research work in hydraulics, especially gravity flow.[1] With the exception of the building, which is of brick, the laboratory is constructed almost entirely of concrete and metal to give it rigidity, permanency, and water-tightness. All water faces of concrete are covered with a 3 to 1 cement-plaster coat three-eighths of an inch thick. Tests have shown the seepage losses to be negligible. The plan and a sectional elevation of the laboratory are shown in figure 1. The circular storage reservoir has a top diameter of 87 feet, side slopes of 1 to 1, and is 6½ feet deep. The headrace connecting it with the weir box is approximately 60 feet long, 4 feet deep, and 6 feet wide for the first 15 feet below the head gates and then expands to 6 feet deep and 10 feet wide at the weir box. The weir box is 20 feet long, 10 feet wide, and 6 feet deep, and has a heavy T-iron frame approximately 3 feet high and 6 feet long in its bulkhead wall. This frame is surfaced, bored for ⅜-inch bolts, and so arranged that the plates containing or forming the notches or orifices and other measuring devices requiring a vertical position can be adjusted accurately for experiments. The joints between the plates and the frame are made

[1] For a complete description of the hydraulic laboratory, see an earlier article by the writer (4).

water-tight by flat rubber gaskets. The water passing through the notches or orifices falls into a concrete spill box 4 feet deep, 10 feet wide, and 9 feet long, which is connected with an auxiliary or waste reser-

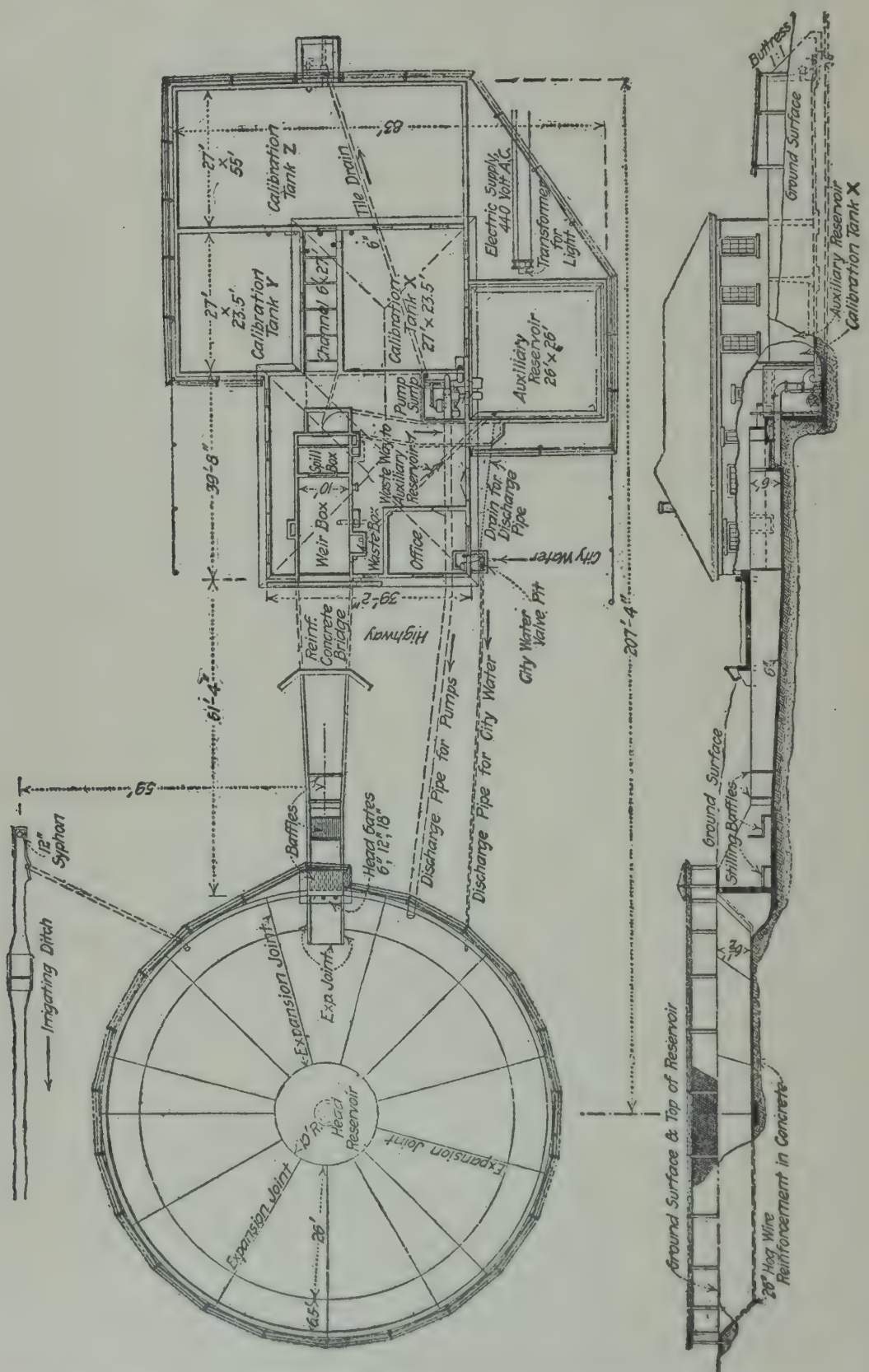

FIG. 1.—Plan and sectional elevations of the Fort Collins hydraulic laboratory.

voir by one channel and with the calibrated tanks by another. The two 22-inch circular openings leading to these channels are separated by a steel plate, and a single disk on the lever arm makes a double shear

gate for the openings. The calibrated tanks and the wasteways on the weir box as well as the spill box are connected with the waste reservoir, from which the water can be returned to the storage reservoir by either a 12-inch or a 5-inch horizontal centrifugal pump driven by electricity. The floors of the calibrated tanks and the waste reservoir are 19 feet lower than the coping of the storage reservoir.

Some of the means used to secure accuracy in the experiments are as follows: The laboratory is so arranged that the centers of the storage reservoir, the headrace, the frame in the end of the weir box, and the channel from the spill box to the calibrated tanks all lie in the same straight line, thus permitting the water to approach and leave the device under experiment in a straight line.

The three head gates between the storage reservoir and the headrace—6, 12, and 18 inches in diameter, respectively—permit a fairly accurate regulation of the water entering the weir box.

Immediately below the head gates a series of two horizontal and two vertical baffles breaks up the eddy currents and reduces pulsations and wave action to such an extent that the water, before entering the weir box, is in a pondlike condition.

In one side of the weir box, about 15 feet upstream from the bulkhead, is an overpour spillway which resembles a door 2 feet high and 3 feet long hinged at the bottom. The top of this spillway when in an upright position is slightly below the top of the weir box. Aprons of oiled canvas attached to the sides of the weir box and to the face of the door prevent leakage and compel the water to pass over the crest of the spillway. A 4-inch gate valve placed at the side of the spillway permits a still more careful regulation of the depth of the water in the weir box. Both the spillway and the gate valve can be adjusted by the hook-gauge observer on the opposite side of the weir box by means of screw controls operated by handwheels placed on the ends of long rods. By always having some water running over the spillway it was possible to keep the head upon the device under test constant throughout the duration of the experiment, usually from 20 to 40 minutes, depending upon the volume of water being run.

The elevations of the water in the weir box and the spill box are observed in concrete gauge boxes built on the outside walls of the respective boxes. These gauge boxes are 1 foot by 2 feet by 4 feet deep, inside dimensions, and the water enters each of them through four 1-inch pipes. The gauge box for the weir box is located 10 feet upstream, and that for the spill box 7 feet downstream from the bulkhead. The pipes leading to the latter, however, take water from the spill box at a point only 3½ feet downstream from the plane of the weir. Each gauge box is equipped with an electric drop light and a Boyden hook gauge anchored in the concrete wall, and readings of the water level can be made to 0.001 of a foot.

In order to refer the elevation of the crest of the notch being experimented with to a reading of the weir-box hook gauge to the nearest 0.001 foot, the instrument shown in figure 2 was devised. The ends of the legs and the hook can be adjusted so as to make the distance from the top of the plate to the groove in the legs exactly equal to the distance from the top of the plate to the point of the hook. By resting the notched legs on the crest of the notch and adjusting the plate to a horizontal position with a sensitive level, the point of the hook is brought to the same elevation as the crest of the notch. Water is run into the weir box, and the surface of the water is adjusted to the point of the crest-hook gauge. Since it is possible to maintain the water level in the weir box quite accurately, the hook-gauge reading in the weir-box gauge box is taken to correspond to the crest elevation of the notch. Repeated determinations of this nature indicated a high degree of accuracy.

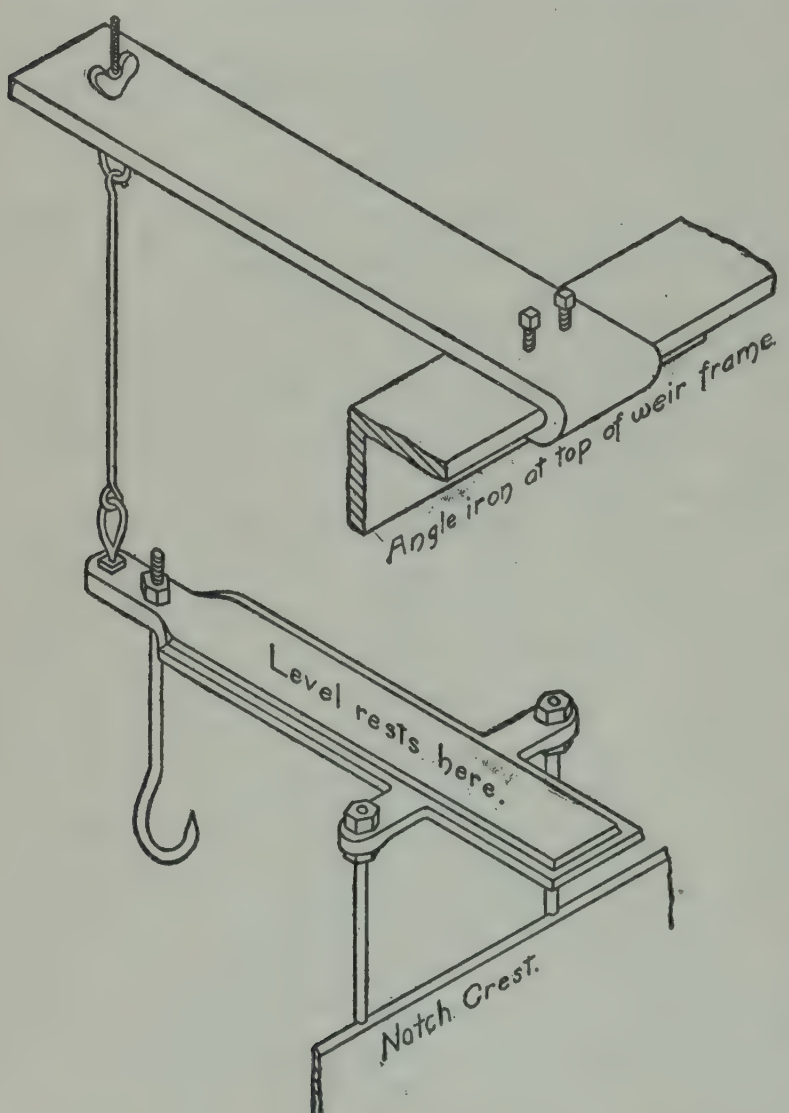

FIG. 2.—Device used in referring elevations of the notch crest to the reading of the hook gauge.

In order to avoid the fluctuating conditions of the flow which occur when tests are being started or stopped, means had to be provided for quickly turning the flow into the channel to the calibrated tanks when the desired conditions for the test had been obtained. This is accomplished by means of the double shear gate used to close the two 22-inch circular openings in the spill box. The lever arm of this gate is 8 feet long, the disk is seated by means of steel shear springs, and the gate is positive and instantaneous in action. When the gate handle reaches midpoint of its swing, it strikes a gong, which is a signal to the hook-gauge observer to start or stop the stop watch used in recording the

duration of the experiments. The error in time in operating the shear gate and the stop watch is only a small fraction of a second.

The calibrated tanks cover an area 55 feet square, divided by 12-inch vertical-sided concrete walls into one tank 27 by 55 feet, two tanks each 23½ by 27 feet, and a channel 6 by 27 feet, which is connected with each tank by a 14-inch circular orifice placed on the floor line and controlled by a gate. The tanks are 8½ feet deep. Their floors are all at the same elevation, and they have a combined capacity of more than 22,000 cubic feet available for experimental purposes. The tanks have been carefully calibrated, corrections having been made for all irregularities, gate openings, rods, etc., and tables have been prepared giving the capacity at each 0.001 foot in elevation. A brass rod 1 inch in diameter and 9 feet long was placed in a vertical position near one corner in each calibrated tank, being held out from the wall about 6 inches by iron brackets set in the concrete (fig. 3). Holes drilled in these rods at carefully measured intervals of about 18 inches serve as datum points when the quantity of water in the tanks is being measured. The elevation of the water in the tanks is determined to 0.001 foot by means of a hook gauge having fixed to its back a heavy clamp provided with a pin which fits snugly into the holes in the rod. A steel ladder was placed adjacent to the brass standard rods in each tank and anchored to the concrete. The platform shown in figure 3 is 20 by 24 inches and can be lowered close to the water surface and secured to any of the ladders by means of hooks. The funnel-shaped arrangement attached to the platform has a ½-inch hole in the bottom and can be adjusted so as to form a stilling basin for the hook gauge. With the water levels at the beginning and the end of the experiment determined by means of the standard rod and hook gauge, the volume run during the experiment can be determined readily from the calibration tables.

Unless otherwise stated, the experiments recorded in this publication were made with notches the edges of which were one-sixteenth inch or less in thickness. The notch plates used were constructed either entirely of brass or of steel with brass notch edges. The crests and sides of the notches were dressed to true angles and straight lines, and by means of a micrometer caliper were calibrated to an allowable divergence of 0.002 inch from a straight line. The triangular notches were dressed to templates. The plate containing the notch under observation was placed in a vertical position in the T frame in the bulkhead of the weir box, and the crests of rectangular and Cipolletti notches were leveled to within 0.001 foot by means of a 12-inch steel-frame level, upon which a bubble division indicated a variation of 0.0004 foot for a length of 1 foot. The inner face of the bulkhead was flush with the crest of the notch. The triangular notch plates were placed so that a vertical line would bisect the angle formed by the sides of the notch. In all the experi-

ments except those upon the effect of contraction (p. 1091) the bottom of the weir box was approximately 4½ feet below the crests of the notches, and the sides of the weir box were 3 to 4½ feet from the ends of the crests, depending upon the size of the notches. In all the experiments the floor

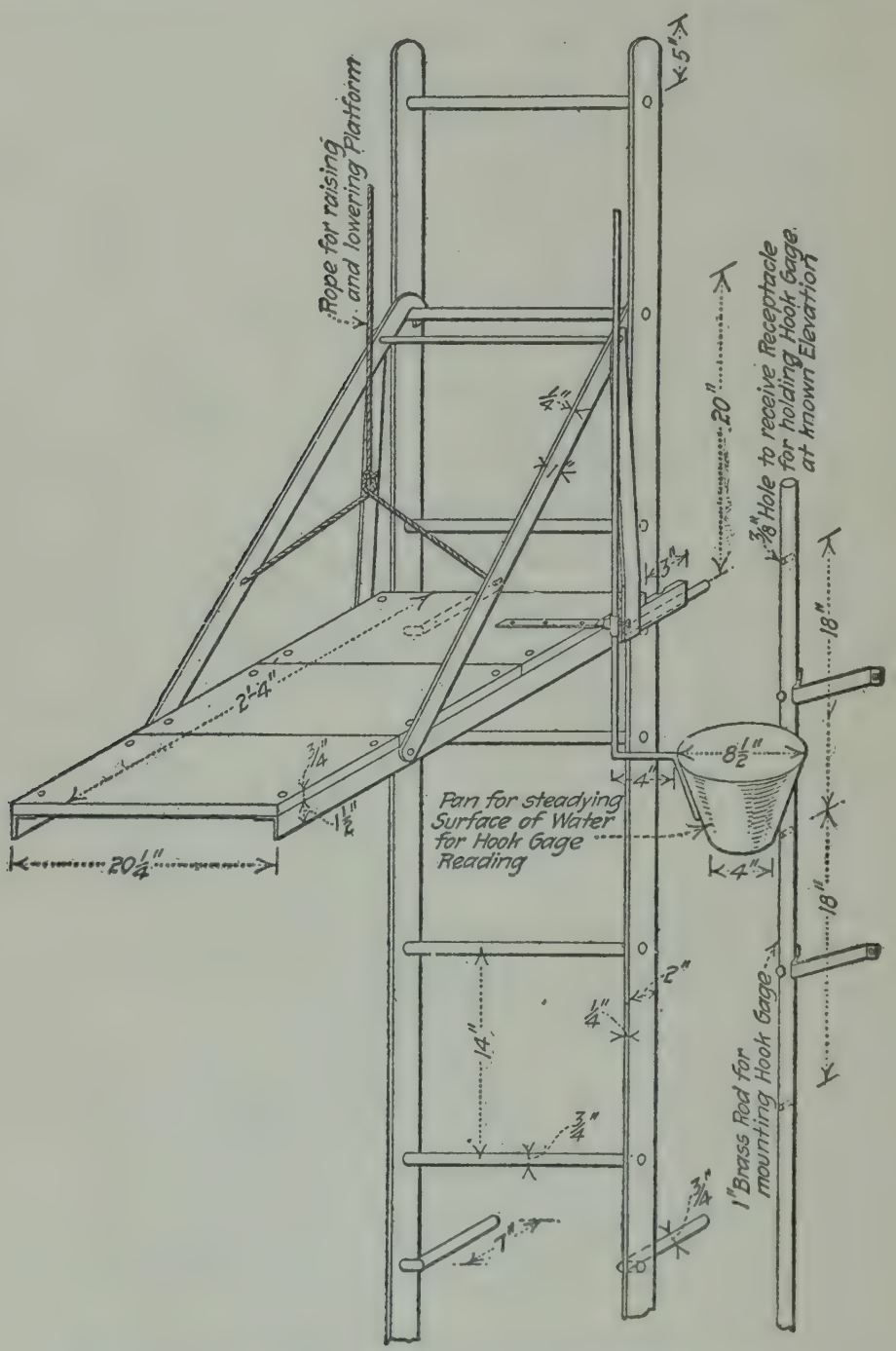

FIG. 3.—Ladder, platform, and datum rod used in calibration tanks.

of the spill box was approximately 4.5 feet below the vertex, or crest, of the notch.

Thirty or forty tests were made upon each notch, the experimental variable being the head. Intervals of head of 0.05 foot were used, and duplicate tests were run for each 0.1 foot of head. If the data from the duplicate tests did not agree within one-half of 1 per cent, the tests were

repeated until such agreement was obtained. It is not claimed that this arbitrary rule insures the accuracy of results of the individual tests, but it did lead to the detection of irregularities in the working conditions and increased the probability of accuracy. Comparatively few tests had to be rerun, which indicates the stability of the experimental tests and the nice control of the heads made possible by the head gates, wasteways, and baffles.

The heads and the corresponding discharges obtained were plotted for the various notches. The curves were then drawn which best represented the discharges through the different notches, the plottings being made upon such a scale that discharge values could be read from the curves to three decimal places.

The following method was used in smoothing the curves and obtaining the values for C in the general formula $Q = CLH^n$:

Discharge values were taken from the curves for each 0.05 foot head, and the slope was determined for each straight line connecting pairs of points. The slope for each point was first taken as the average between the slopes of the two straight lines to which it was common; then, calling the point in question b, the point for the next 0.05 foot head above, a, and that below, c, the slopes were given a second smoothing by the equation $\frac{a+2b+c}{4}=b$; and a third smoothing was obtained by substituting the values obtained by the second smoothing in the equation $\frac{a+2b+3c+2d+e}{9}=c$. These values were plotted, and the equation of the resulting curve was used to compute the last smoothing of the slopes. Substituting these computed values for n in the general formula $Q = CLH^n$, the corresponding value of C was obtained for each head.

EXPERIMENTS WITH NOTCHES HAVING FREE FLOW

DEDUCTIONS OF FORMULAS FOR RECTANGULAR AND TRAPEZOIDAL NOTCHES

The general type of formula heretofore used for discharges through rectangular and trapezoidal notches is $Q = CLH^n$, in which L is length of crest, H the head of water over the crest, and C and n are constant for each type of weir. Expressed logarithmically, the general formula becomes $\log Q = \log C + \log L + n \log H$, which equation, when plotted, gives a straight line whose slope is n and whose intercept is $\log C + \log L$.

The data obtained for the rectangular and Cipolletti notches, when plotted logarithmically, gave curves instead of straight lines. It was found, however, that a general straight-line equation could be deduced for the discharges through the rectangular notches, which, within the range of the experiments, would give discharges as close to the experi-

mental data as would the general curve equation. The experimental data indicate, however, that the general curve equation would hold true for a greater range of notch lengths and heads than would the general straight-line equation. Table I, for the Cipolletti notches, gives the discharge values for the different heads as read from the curve, the experimental discharge values (observed discharges) at greatest variance with the curve discharge values, and the values of the exponents n and coefficients C necessary in the Cipolletti formula to give the discharges obtained in the experiments. The values of n and C in the table show that the discharges for any notch, if plotted logarithmically, would not give a straight line, since neither the n's nor the C's are constant. A comparison of the curve discharge values and the observed discharges in the table also serves to indicate the accuracy of the experimental data. The variations of the n's and C's also hold for the rectangular notches, but are not so pronounced as in the case of the Cipolletti notches, since the discharge curves for rectangular notches are flatter.

TABLE I.—*Discharges through Cipolletti notches, and the exponents and coefficients necessary in using the Cipolletti formula*

Head.	0.50062-foot notch.				1.0050-foot notch.				1.5028-foot notch.				2.0002-foot notch.				3.0011-foot notch.				4.0086-foot notch.			
	Discharge, cubic feet per second.		(*n*)	(*C*)	Discharge, cubic feet per second.		(*n*)	(*C*)	Discharge, cubic feet per second.		(*n*)	(*C*)	Discharge, cubic feet per second.		(*n*)	(*C*)	Discharge, cubic feet per second.		(*n*)	(*C*)	Discharge, cubic feet per second.		(*n*)	(*C*)
	Observed.	Curve.			Observed.	Curve.			Observed.	Curve.			Observed.	Curve.			Observed.	Curve.			Observed.	Curve.		
Feet.																								
0.20	0.152	0.149	1.530	3.492	0.300	0.300	1.498	3.327	0.459	0.455	1.486	3.309	0.603	0.602	1.480	3.255	0.909	0.902	1.473	3.217	1.200	1.206	1.470	3.205
.30	.287	.284	1.565	3.733	.554	.554	1.517	3.424	.829	.829	1.499	3.353	1.100	1.100	1.490	3.307	1.633	1.648	1.480	3.263	2.200	2.191	1.476	3.232
.40	.453	.452	1.600	3.911	.866	.865	1.536	3.516	1.289	1.282	1.513	3.413	1.693	1.695	1.501	3.353	2.538	2.538	1.487	3.303	3.342	3.365	1.481	3.261
.50	.653	.655	1.636	4.066	1.216	1.217	1.555	3.558	1.801	1.798	1.526	3.446	2.323	2.373	1.511	3.381	3.528	3.528	1.495	3.314	4.700	4.700	1.487	3.287
.60	.890	.890	1.671	4.174	1.626	1.622	1.574	3.608	2.378	2.375	1.539	3.469	3.146	3.142	1.521	3.416	4.663	4.634	1.502	3.326	6.172	6.180	1.493	3.305
.70	1.158	1.159	1.706	4.254	2.079	2.072	1.593	3.639	3.018	3.013	1.553	3.488	3.952	3.952	1.532	3.412	5.890	5.870	1.509	3.350	7.807	7.795	1.499	3.319
.80	1.455	1.455	1.742	4.284	2.565	2.562	1.612	3.653	3.718	3.708	1.566	3.500	4.836	4.845	1.542	3.417	7.173	7.190	1.516	3.360	9.532	9.532	1.504	3.326
.90	1.784	1.786	1.778	4.303	3.109	3.111	1.631	3.676	4.458	4.463	1.579	3.508	5.815	5.814	1.553	3.424	8.574	8.574	1.523	3.354	11.342	11.390	1.510	3.331
1.00	2.149	2.155	.....	.....	3.693	3.696	1.650	3.678	5.262	5.262	1.592	3.502	6.792	6.844	1.563	3.422	10.081	10.080	1.530	3.359	13.376	13.376	1.515	3.337
1.10	.....	.....	.....	.....	4.332	4.340	.....	.....	6.138	6.134	1.605	3.503	7.943	7.941	1.573	3.417	11.655	11.655	1.538	3.354	15.467	15.425	1.521	3.329
1.20	.....	.....	.....	.....	.....	.....	.....	.....	7.062	7.060	1.618	3.498	9.116	9.107	1.584	3.411	13.347	13.355	1.545	3.357	.....	.....	.....	.....
1.30	.....	.....	.....	.....	.....	.....	.....	.....	8.022	8.035	1.632	3.484	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

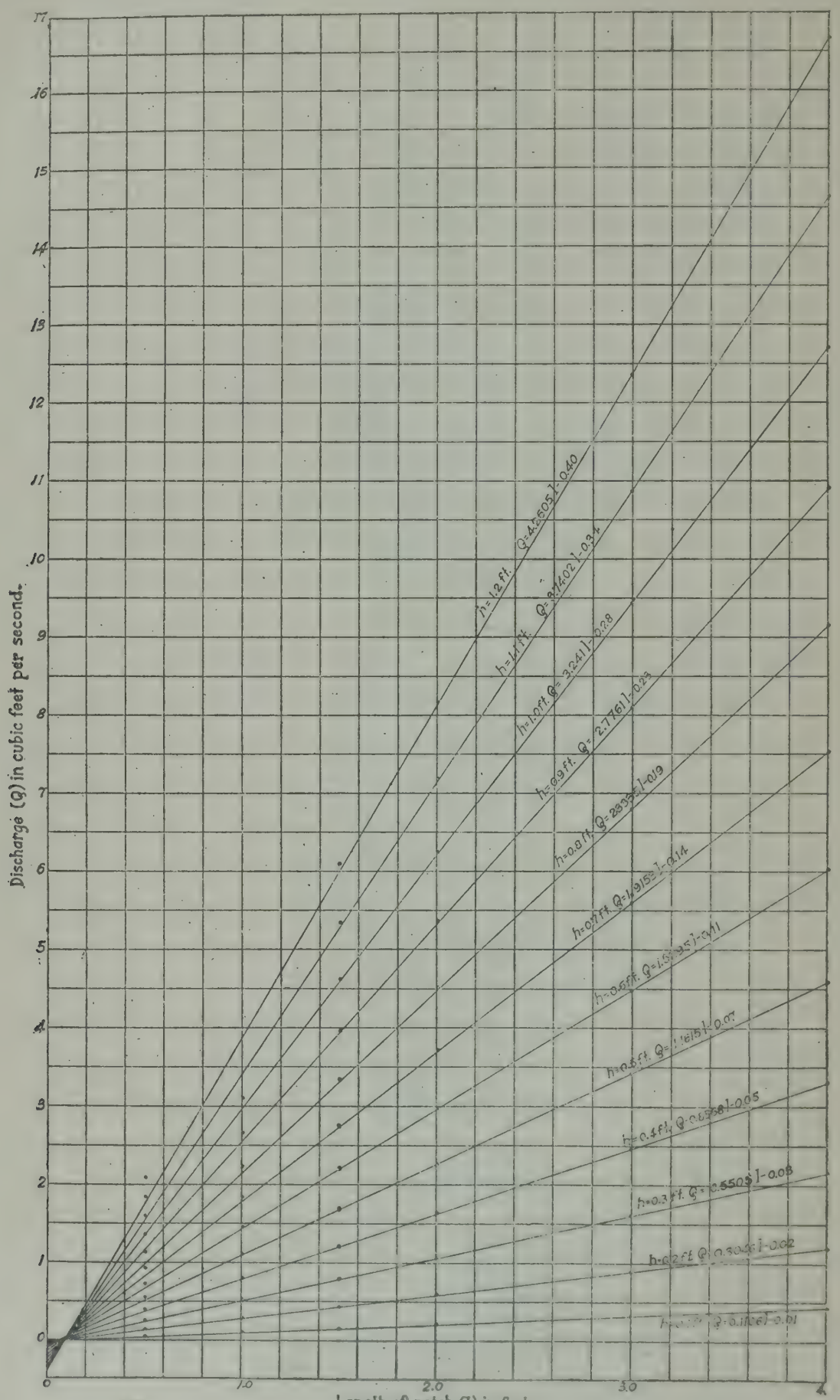

FIG. 4.—Curves showing the relation between discharges with constant heads through rectangular notches of different lengths and the lengths of the notches.

RECTANGULAR NOTCHES

With rectangular notches 226 tests were made, the actual crest lengths used being 0.50721 foot, 1.0055 feet, 1.5026 feet, 2.0057 feet, 2.9970 feet, and 4.0065 feet. These actual lengths were used in all computations connected with the derivation of the formula.

DERIVATION OF THE FORMULA

The discharge values for 0.05-foot increments of head, taken from the curves plotted from the experimental data, were used in the following deductions, thereby eliminating to a large extent the experimental

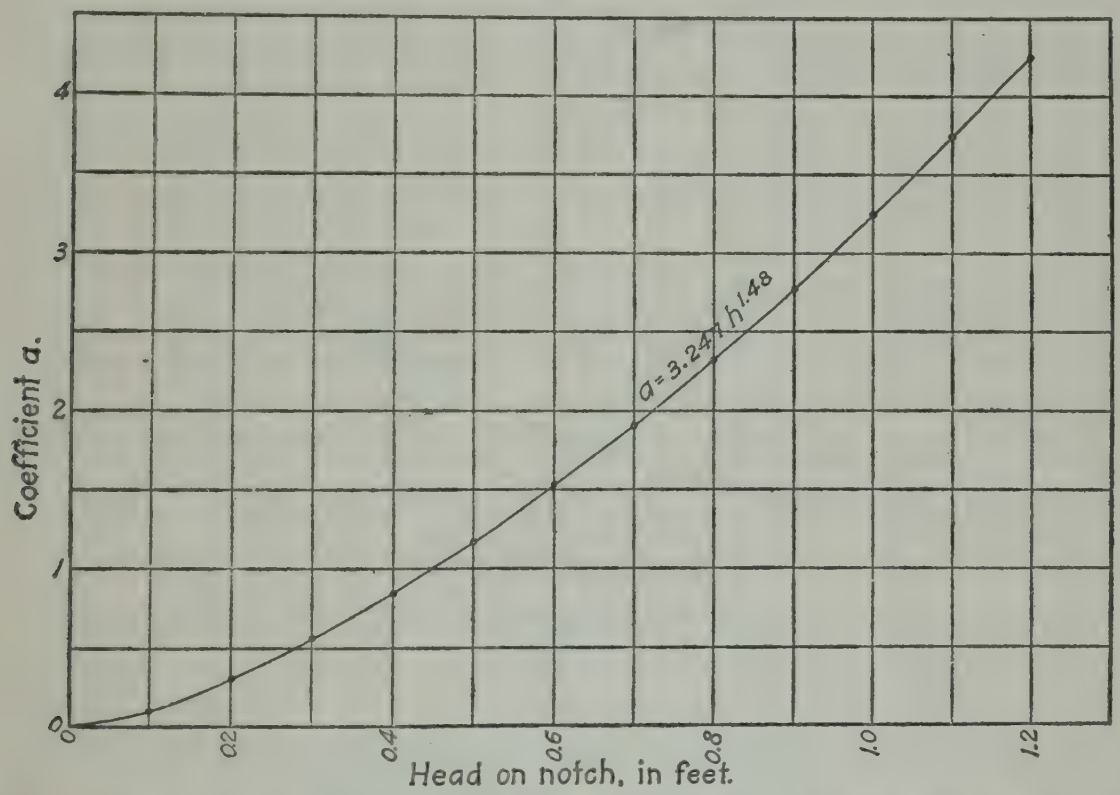

FIG. 5.—Curve showing the relation between a in the equation $Q=aL-b$ and the heads on rectangular notches.

irregularities. The discharge values for the different notches were plotted (fig. 4) with the lengths of crests (L) as abscissas, and the discharges (Q) as ordinates. A straight line was then drawn for each head by passing it through the points representing the discharges over the 3- and 4-foot crests with the given head. The equations of these straight lines were found to be of the form $Q=aL-b$.

The slopes (a) of the lines were computed from the coordinates of the discharge values with the 3- and 4-foot crests. The relations between the heads (H) and the slopes (a) in the above formula were plotted (fig. 5) and gave a curve the equation for which was found to be $a=3.247H^{1.48}$.

The relations between the heads (H) and the intercepts (b) in the equation $Q=aL-b$ are shown in figure 6. The equation for the curve was found to be $b=0.283H^{1.9}$.

The offsets from each of the straight lines in figure 4 to the points representing the discharges with the head for which the line was drawn were tabulated, and an expression for the offsets was determined to be $\frac{0.283H^{1.9}}{1+2L^{1.8}}$.

Substituting the values of a and b in the equation form $Q=aL-b$ and making a correction for the offsets from the straight lines, the formula for the rectangular notches was found to be

$$Q=3.247\ LH^{1.48}-\left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9}$$

Table II gives the discharge values for the rectangular notches of different lengths computed by this formula. This formula gives discharge

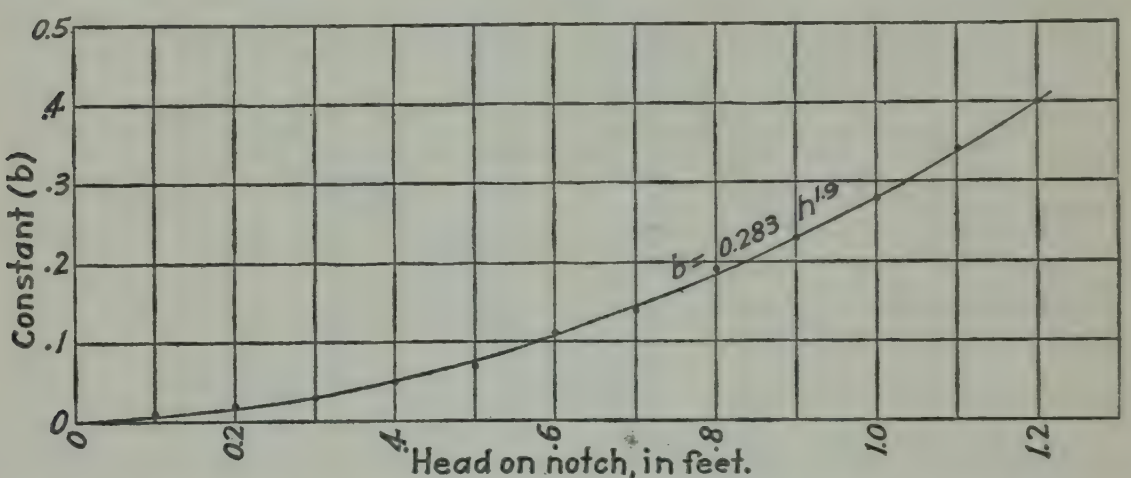

FIG. 6.—Curve showing the relation between b in the equation $Q=aL-b$ and the heads on rectangular notches.

values within a maximum of approximately 1.2 per cent of the values indicated on the curves plotted from the experimental data, but the average variation is only 0.28 per cent. Table V compares the values indicated on the curves plotted from the experimental data and values computed with formulas.

TABLE II.—*Discharges (in cubic feet per second) through rectangular weir notches* [1]

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.20	2⅜	0.291	0.439	0.588	0.887	1.19
.21	2½	.312	.472	.632	.954	1.28
.22	2⅝	.335	.505	.677	1.02	1.37
.23	2¾	.358	.539	.723	1.09	1.46
.24	2⅞	.380	.574	.769	1.16	1.55

[1] Computed by the formula $Q=3.247LH^{1.48}-\left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9}$

TABLE II.—*Discharges (in cubic feet per second) through rectangular weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.25	3	0.404	0.609	0.817	1.23	1.65
.26	3 1/8	.428	.646	.865	1.31	1.75
.27	3 1/4	.452	.682	.914	1.38	1.85
.28	3 3/8	.477	.720	.965	1.46	1.95
.29	3 1/2	.502	.758	1.02	1.53	2.05
.30	3 5/8	.527	.796	1.07	1.61	2.16
.31	3 3/4	.553	.836	1.12	1.69	2.27
.32	3 13/16	.580	.876	1.18	1.77	2.37
.33	3 15/16	.606	.916	1.23	1.86	2.48
.34	4 1/16	.634	.957	1.28	1.94	2.60
.35	4 3/16	.661	.999	1.34	2.02	2.71
.36	4 5/16	.688	1.04	1.40	2.11	2.82
.37	4 7/16	.717	1.08	1.45	2.20	2.94
.38	4 9/16	.745	1.13	1.51	2.28	3.06
.39	4 11/16	.774	1.17	1.57	2.37	3.18
.40	4 13/16	.804	1.21	1.63	2.46	3.30
.41	4 15/16	.833	1.26	1.69	2.55	3.42
.42	5 1/16	.863	1.30	1.75	2.65	3.54
.43	5 3/16	.893	1.35	1.81	2.74	3.67
.44	5 1/4	.924	1.40	1.88	2.83	3.80
.45	5 3/8	.955	1.44	1.94	2.93	3.93
.46	5 1/2	.986	1.49	2.00	3.03	4.05
.47	5 5/8	1.02	1.54	2.07	3.12	4.18
.48	5 3/4	1.05	1.59	2.13	3.22	4.32
.49	5 7/8	1.08	1.64	2.20	3.32	4.45
.50	6	1.11	1.68	2.26	3.42	4.58
.51	6 1/8	1.15	1.73	2.33	3.52	4.72
.52	6 1/4	1.18	1.78	2.40	3.62	4.86
.53	6 3/8	1.21	1.84	2.46	3.73	4.99
.54	6 1/2	1.25	1.89	2.53	3.83	5.13
.55	6 5/8	1.28	1.94	2.60	3.94	5.27
.56	6 3/4	1.31	1.99	2.67	4.04	5.42
.57	6 13/16	1.35	2.04	2.74	4.15	5.56
.58	6 15/16	1.38	2.09	2.81	4.26	5.70
.59	7 1/16	1.42	2.15	2.88	4.36	5.85
.60	7 3/16	1.45	2.20	2.96	4.47	6.00
.61	7 5/16	1.49	2.25	3.03	4.58	6.14
.62	7 7/16	1.52	2.31	3.10	4.69	6.29
.63	7 9/16	1.56	2.36	3.17	4.81	6.44
.64	7 11/16	1.60	2.42	3.25	4.92	6.59
.65	7 13/16	1.63	2.47	3.33	5.03	6.75
.66	7 15/16	1.67	2.53	3.40	5.15	6.90
.67	8 1/16	1.71	2.59	3.48	5.26	7.05
.68	8 3/16	1.74	2.64	3.56	5.38	7.21
.69	8 1/4	1.78	2.70	3.63	5.49	7.36
.70	8 3/8	1.82	2.76	3.71	5.61	7.52
.71	8 1/2	1.86	2.81	3.78	5.73	7.68
.72	8 5/8	1.90	2.87	3.86	5.85	7.84
.73	8 3/4	1.93	2.93	3.94	5.97	8.00
.74	8 7/8	1.97	2.99	4.02	6.09	8.17

TABLE II.—*Discharges (in cubic feet per second) through rectangular weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.75	9	2.01	3.05	4.10	6.21	8.33
.76	9 1/8	2.05	3.11	4.18	6.33	8.49
.77	9 1/4	2.09	3.17	4.26	6.45	8.66
.78	9 3/8	2.13	3.23	4.34	6.58	8.82
.79	9 1/2	2.17	3.29	4.42	6.70	8.99
.80	9 5/8	2.21	3.35	4.51	6.83	9.16
.81	9 3/4	2.25	3.41	4.59	6.95	9.33
.82	9 13/16	2.29	3.47	4.67	7.08	9.50
.83	9 15/16	2.33	3.54	4.75	7.21	9.67
.84	10 1/16	2.37	3.60	4.84	7.33	9.84
.85	10 3/16	2.41	3.66	4.92	7.46	10.01
.86	10 5/16	2.46	3.72	5.01	7.59	10.19
.87	10 7/16	2.50	3.79	5.10	7.72	10.36
.88	10 9/16	2.54	3.85	5.18	7.85	10.54
.89	10 11/16	2.58	3.92	5.27	7.99	10.71
.90	10 13/16	2.62	3.98	5.35	8.12	10.89
.91	10 15/16	2.67	4.05	5.44	8.25	11.07
.92	11 1/16	2.71	4.11	5.53	8.38	11.25
.93	11 3/16	2.75	4.18	5.62	8.52	11.43
.94	11 1/4	2.79	4.24	5.71	8.65	11.61
.95	11 3/8	2.84	4.31	5.80	8.79	11.79
.96	11 1/2	2.88	4.37	5.89	8.93	11.98
.97	11 5/8	2.93	4.44	5.98	9.06	12.16
.98	11 3/4	2.97	4.51	6.07	9.20	12.34
.99	11 7/8	3.01	4.57	6.15	9.34	12.53
1.00	12	3.06	4.64	6.25	9.48	12.72
1.01	12 1/8	.....	4.71	6.34	9.62	12.91
1.02	12 1/4	.....	4.78	6.43	9.76	13.10
1.03	12 3/8	.....	4.85	6.52	9.90	13.28
1.04	12 1/2	.....	4.92	6.62	10.04	13.47
1.05	12 5/8	.....	4.98	6.71	10.18	13.66
1.06	12 3/4	.....	5.05	6.80	10.32	13.85
1.07	12 13/16	.....	5.12	6.90	10.46	14.04
1.08	12 15/16	.....	5.19	6.99	10.61	14.24
1.09	13 1/16	.....	5.26	7.09	10.75	14.43
1.10	13 3/16	.....	5.34	7.19	10.90	14.64
1.11	13 5/16	.....	5.41	7.28	11.04	14.83
1.12	13 7/16	.....	5.48	7.38	11.19	15.03
1.13	13 9/16	.....	5.55	7.47	11.34	15.22
1.14	13 11/16	.....	5.62	7.57	11.49	15.42
1.15	13 13/16	.....	5.69	7.66	11.64	15.62
1.16	13 15/16	.....	5.77	7.76	11.79	15.82
1.17	14 1/16	.....	5.84	7.86	11.94	16.02
1.18	14 3/16	.....	5.91	7.96	12.09	16.23
1.19	14 1/4	.....	5.98	8.06	12.24	16.43
1.20	14 3/8	.....	6.06	8.16	12.39	16.63
1.21	14 1/2	.....	6.13	8.26	12.54	16.83
1.22	14 5/8	.....	6.20	8.36	12.69	17.04
1.23	14 3/4	.....	6.28	8.46	12.85	17.25
1.24	14 7/8	.....	6.35	8.56	12.99	17.45

TABLE II.—*Discharges (in cubic feet per second) through rectangular weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
1.25	15	.....	6.43	8.66	13.14	17.66
1.26	15 1/8	.....	.....	.....	13.30	17.87
1.27	15 1/4	.....	.....	.....	13.45	18.07
1.28	15 3/8	.....	.....	.....	13.61	18.28
1.29	15 1/2	.....	.....	.....	13.77	18.50
1.30	15 5/8	.....	.....	.....	13.93	18.71
1.31	15 3/4	.....	.....	.....	14.09	18.92
1.32	15 13/16	.....	.....	.....	14.24	19.13
1.33	15 15/16	.....	.....	.....	14.40	19.34
1.34	16 1/16	.....	.....	.....	14.56	19.55
1.35	16 3/16	.....	.....	.....	14.72	19.77
1.36	16 5/16	.....	.....	.....	14.88	19.98
1.37	16 7/16	.....	.....	.....	15.04	20.20
1.38	16 9/16	.....	.....	.....	15.20	20.42
1.39	16 11/16	.....	.....	.....	15.36	20.64
1.40	16 13/16	.....	.....	.....	15.53	20.86
1.41	16 15/16	.....	.....	.....	15.69	21.08
1.42	17 1/16	.....	.....	.....	15.85	21.30
1.43	17 3/16	.....	.....	.....	16.02	21.52
1.44	17 1/4	.....	.....	.....	16.19	21.74
1.45	17 3/8	.....	.....	.....	16.34	21.96
1.46	17 1/2	.....	.....	.....	16.51	22.18
1.47	17 5/8	.....	.....	.....	16.68	22.41
1.48	17 3/4	.....	.....	.....	16.85	22.63
1.49	17 7/8	.....	.....	.....	17.01	22.85
1.50	18	.....	.....	.....	17.18	23.08

The discharges through a notch having a crest length of 0.5 foot did not follow the same law as those through larger notches. This was probably owing to the greater effect of friction in the smaller notch and to the interference due to the end-contraction filaments of flow crossing each other in the middle of the notch section. The formula

$$Q = 1.593 H^{1.526}\left(1 + \frac{1}{800 H^{2.3}}\right)$$

was found to give discharge values consistent with the curve plotted from experimental data for the 0.5-foot notch. The use of such a notch is very limited, and the 90° triangular notch is as accurate and much more satisfactory.

COMPARISON OF THE FRANCIS FORMULA AND THE NEW FORMULA

The discharge values obtained for rectangular notches by the Francis and the new formulas are shown in graphic form in figure 7 and in tabular form in Table III.

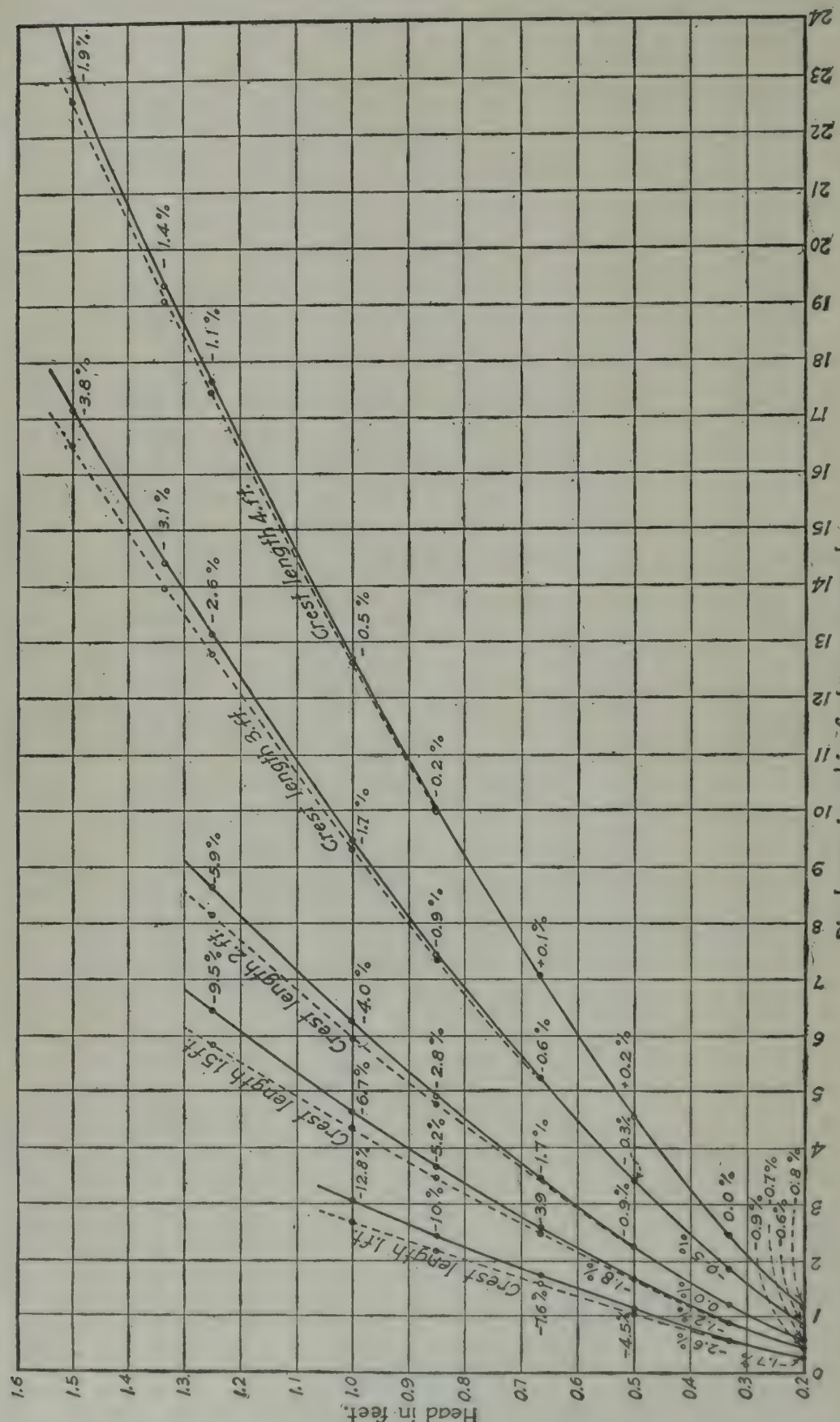

FIG. 7.—Curves showing discharges through rectangular notches of different lengths.

TABLE III.—*Comparison of discharges through rectangular notches computed from the Francis formula and the new formula*

Head.	1-foot crest.			1½-foot crest.			2-foot crest.			3-foot crest.			4-foot crest.		
	Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula.[1]		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula.[1]		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula.[1]		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula.[1]		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula.[1]	
		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.
Feet.															
0.20	0.291	0.286	98.3	0.439	0.435	99.1	0.588	0.584	99.3	0.887	0.882	99.4	1.19	1.18	99.2
.33	.606	.590	97.4	.916	.905	98.8	1.22	1.22	100.0	1.86	1.85	99.5	2.48	2.48	100.0
.50	1.11	1.06	95.5	1.68	1.65	98.2	2.26	2.24	99.1	3.42	3.41	99.7	4.58	4.59	100.2
.67	1.71	1.58	92.4	2.59	2.49	96.1	3.47	3.41	98.3	5.26	5.23	99.4	7.05	7.06	100.1
.85	2.41	2.17	90.0	3.66	3.47	94.8	4.92	4.78	97.2	7.45	7.38	99.1	10.01	9.99	99.8
1.00	3.06	2.67	87.2	4.64	4.33	93.3	6.24	5.99	96.0	9.48	9.32	98.3	12.72	12.65	99.5
1.25	.....	.....	.....	6.43	5.82	90.5	8.65	8.14	94.1	13.14	12.80	97.4	17.65	17.45	98.9
1.33	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.40	13.96	96.9	19.34	19.07	98.6
1.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	17.17	16.52	96.2	23.08	22.64	98.1

[1] The Francis formula: $Q=3.33(L-.2H)H^{3/2}$.

The curves and Table III show that except for a small range of heads on the 4-foot notch the discharges computed by the Francis formula are too small. The actual discharges, however, where the head did not exceed one-third of the length of the crest, did not vary much from those computed by the Francis formula and support the statement of Francis that his formula would give discharge values correct to within 2 per cent, provided the head does not exceed one-third the length of the crest. Nevertheless the fact that the curves plotted from the experimental data have no sudden breaks or changes of direction shows that no limit need be placed upon the head, provided the proper formula is used to compute the discharge. It also shows that the necessity of the limit on the application of the Francis formula was due to the mathematical shortcoming of the formula and not to any peculiarity inherent in the rectangular notch. The new formula not only gives greater accuracy within the range of the Francis formula but also permits the accurate measurement of discharges with the heads exceeding one-third the length of the crest. The maximum limit of the ratio of the head to the crest length with the new formula has not been ascertained, the greatest ratio experimented with being 1 to 1 with the 1-foot notch. The parts of all the curves showing the discharges with higher heads, however, were quite consistent in all cases with the rest of the curves. A head of 1 foot was run over a 0.5-foot notch, but the results were inconclusive, as the discharges through the 0.5-foot notch do not follow the general formula.

The new formula is more complicated than the Francis formula, but gives discharge values which are more accurate within the limits of these experiments, and since tables are generally consulted to determine the flow that is passing through a notch, the practical disadvantage of the new formula is largely overcome. If one is obliged to use a formula in the field for computing the discharge, an approximation usually is sufficient, and the Francis formula gives discharges sufficiently accurate for practical needs.

Straight-line Formula

As stated on page 1059, it was found, when the experimental data for the rectangular notches were plotted logarithmically, that a general straight-line formula could be deduced which, within the range of the experiments, would give discharge values as close to the plotted values as did the general formula deduced above. The equations for the straight lines best representing the discharges with the given heads through the different notches were found to be as shown in Table IV.

TABLE IV.—*Equations for straight lines representing discharges through rectangular weir notches*

Length of crest.	Equations of line.
Feet.	
1.0055	$Q=3.078LH^{1.463}$
1.5026	$Q=3.106LH^{1.465}$
2.0057	$Q=3.125LH^{1.466}$
2.9970	$Q=3.154LH^{1.467}$
4.0056	$Q=3.172LH^{1.473}$

The coefficient values (C) in the above equations were plotted (fig. 8) against the lengths of crests (L), and the exponent values

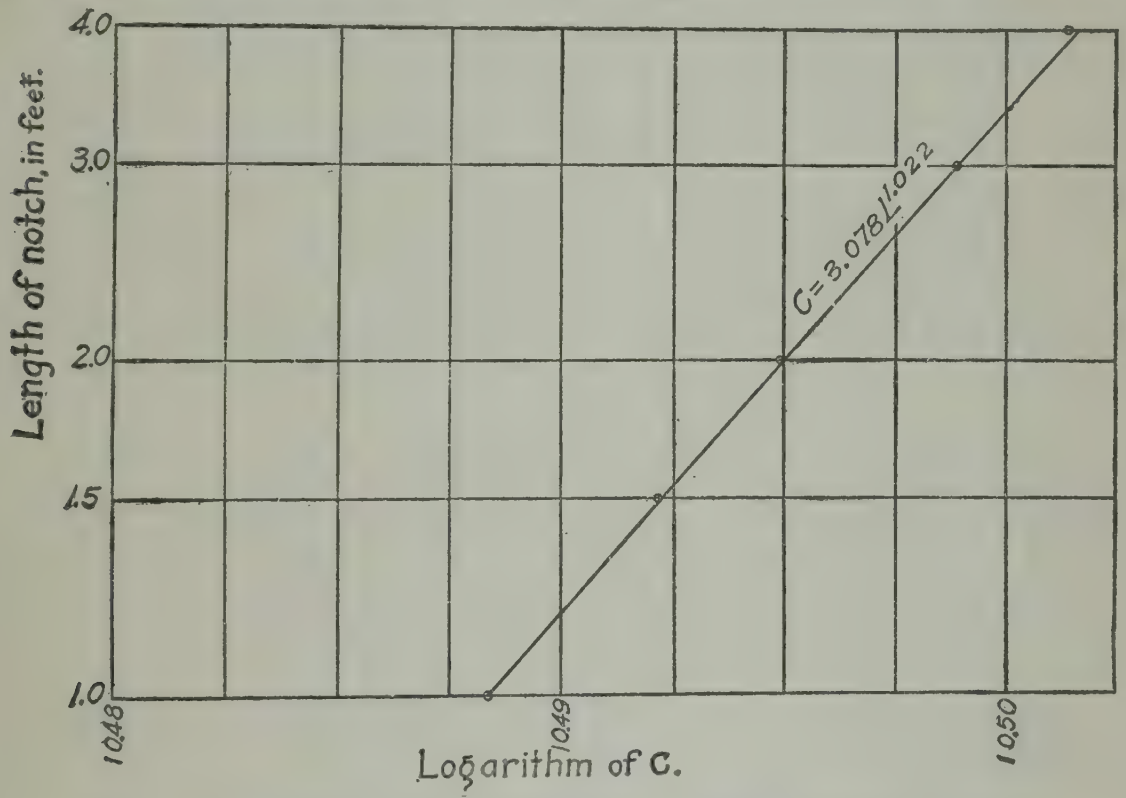

FIG. 8.—Curve showing relation of coefficients (C) to lengths of rectangular notches.

(n) were plotted (fig. 9) against the lengths of crests (L). Average straight lines drawn to represent the points were found to have the equations $C=3.078L^{1.022}$ and $n=1.46+0.003L$.

Substituting these values of C and n in the equation $Q=CLH^n$, the formula for the discharge through rectangular notches was found to be

$$Q=3.08L^{1.022}H^{(1.46+.003L)}.$$

This formula gives discharge values that agree within a maximum of 0.7 per cent with the values indicated on the curves plotted from the experimental data, but the average variation is only 0.26 per cent.

Table V gives the discharges through the notches used, computed by the curve and by the straight-line formulas, also the values indicated on the curves plotted from the experimental data.

TABLE V.—*Discharges (in cubic feet per second) for rectangular notches as shown by curves plotted from experimental data, and discharges computed by curve and straight-line formulas*

Head.	1.0055-foot notch.			1.5026-foot notch.			2.0057-foot notch.			2.997-foot notch.			4.0056-foot notch.		
	Experimental data.	Curve formula.	Straight-line formula.	Experimental data.	Curve formula.	Straight-line formula.	Experimental data.	Curve formula.	Straight-line formula.	Experimental data.	Curve formula.	Straight-line formula.	Experimental data.	Curve formula.	Straight-line formula.
Feet.															
0.2	0.293	0.293	0.294	0.443	0.440	0.442	0.593	0.590	0.593	0.890	0.886	0.889	1.194	1.189	1.190
.3	.531	.530	.532	.800	.797	.801	1.079	1.071	1.074	1.617	1.610	1.613	2.163	2.161	2.162
.4	.806	.808	.811	1.220	1.217	1.220	1.640	1.635	1.637	2.461	2.462	2.461	3.302	3.304	3.302
.5	1.115	1.120	1.123	1.680	1.688	1.692	2.267	2.268	2.271	3.411	3.418	3.416	4.594	4.589	4.585
.6	1.459	1.462	1.467	2.195	2.205	2.210	2.969	2.964	2.966	4.474	4.470	4.465	6.013	6.004	5.997
.7	1.834	1.830	1.838	2.755	2.761	2.770	3.718	3.716	3.719	5.595	5.605	5.600	7.532	7.533	7.524
.8	2.233	2.223	2.235	3.354	3.357	3.368	4.519	4.519	4.523	6.795	6.821	6.814	9.157	9.171	9.156
.9	2.660	2.639	2.655	3.988	3.987	4.002	5.367	5.369	5.375	8.090	8.110	8.101	10.910	10.906	10.892
1.0	3.103	3.076	3.097	4.664	4.650	4.670	6.238	6.265	6.273	9.432	9.467	9.457	12.706	12.734	12.720
1.1	.....	.....	.....	5.370	5.346	5.369	7.190	7.205	7.214	10.866	10.893	10.878	14.642	14.656	14.635
1.2	.....	.....	.....	6.133	6.068	6.099	8.174	8.181	8.195	12.356	12.374	12.361	16.666	16.653	16.635
1.3	.....	.....	.....	6.903	6.819	6.857	9.193	9.196	9.215	13.876	13.918	13.903	.....	.....	.....

In locating the straight lines on the logarithmic plot, it was found that the points for the 1.0055-foot notch could be covered quite closely by three straight lines approximately equal in length. The same was approximately true of the points for the 1.5026-foot notch. Only two

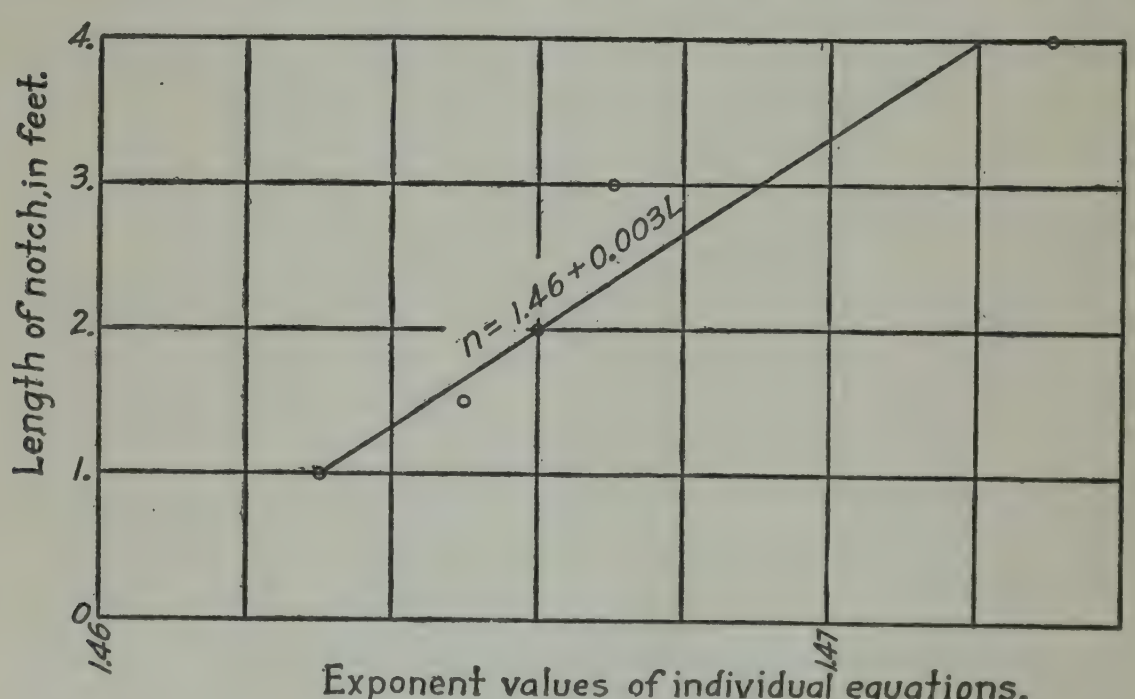

FIG. 9.—Curve showing relation of n to length of rectangular notches.

straight lines each, however, were required for the 2.0057-foot and 2.997-foot notches, although a third could be assumed near the upper part of the curves in each case. For the 4.0056-foot notch there was only one point of change, and it was well above the middle of the curve. These facts indicate that had large enough heads been run on the longer notches to give the same ratio of length of crest to head as was obtained with the

1-foot notch, an equal number of lines would have been required to cover the points. If a single straight line is taken to represent the discharge curve, and it is placed to represent best the discharges with the lower heads, as was done above, the part of the true discharge curve for the higher heads diverges rapidly from the straight line. The curve formula takes account of the law of variation of the discharge curves better than does the straight-line formula, and, consequently, it appears that it will give closer values for the higher heads and for longer notches than those experimented with.

The straight-line equation for the 0.5-foot notch was found to be $Q = 1.566H^{1.504}$.

This equation was found to give discharge values within approximately 1 per cent of the values indicated on the curve plotted from the experimental data.

CIPOLLETTI NOTCHES

With notches having side slopes of one horizontal to four vertical, 219 tests were made. The actual crest lengths used were 0.50062 foot, 1.0050 feet, 1.5028 feet, 2.0002 feet, 3.0011 feet, and 4.0058 feet, respectively, and these lengths were used throughout the following calculations.

DERIVATION OF THE FORMULA

The difference between the areas of a Cipolletti and a rectangular notch with equal crest length is the area of a 28° 4′ (approximately) triangular notch—that is, one having one to four side slopes. It was found, however, that the discharges through such a notch (see Table X) with a given head did not exactly equal the difference between the discharges through a rectangular and a Cipolletti notch with equal crest lengths and the same head. While the differences between the discharges through the Cipolletti and rectangular notches increase with the head for all crest lengths, there was no regular increase or decrease in the differences in the discharges with increases in the crest lengths so long as the heads were less than approximately 0.8 foot, but for higher heads the differences in discharges decreased as the crest lengths increased. The comparison of the differences is very unreliable for heads as low as 0.2 or 0.3 foot. The discharges through the 28° 4′ notch are greater than the differences between the discharges of the Cipolletti and rectangular notches for all heads up to approximately 2.5 feet, the percentages of excess decreasing with the increases in head and equaling zero with a head of approximately 2.5.

The differences between the discharges through the rectangular and Cipolletti notches for each of the crest lengths were determined from the curves plotted from the experimental data and an average made for each 0.1 foot of head. These averages were then plotted logarithmically against the head, and the equation of the curve representing the differ-

ence in discharge was found to be $D = .609H^{2.5}$. By adding the term $.609H^{2.5}$ to the general formula for discharges through rectangular notches (page 1064), the general formula for discharges through Cipolletti notches was found to be

$$Q = 3.247LH^{1.48} - \left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9} + 0.609H^{2.5}$$

This formula gives discharge values for 1-, 1½-, 2-, 3-, and 4-foot notches that agree within 0.5 per cent of the values indicated on the curves plotted from the experimental data, except for the lower heads on the 1-foot notch, where the maximum discrepancy, owing to the small discharge, is approximately 1½ per cent. The discrepancies are positive in some cases and negative in others. (See Table VII for discharge values indicated by the curves plotted from the experimental data and discharge values computed by the formulas.)

Table VI gives the discharge values for Cipolletti notches of different lengths computed by the new formula.

TABLE VI.—*Discharges (in cubic feet per second) through Cipolletti weir notches*[1]

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.20	2 3/8	0.30	0.45	0.60	0.90	1.20
.21	2 1/2	.32	.48	.64	.97	1.29
.22	2 5/8	.35	.52	.69	1.04	1.38
.23	2 3/4	.37	.55	.74	1.11	1.47
.24	2 7/8	.39	.59	.79	1.18	1.57
.25	3	.42	.63	.84	1.25	1.67
.26	3 1/8	.45	.67	.89	1.33	1.77
.27	3 1/4	.47	.71	.94	1.40	1.87
.28	3 3/8	.50	.75	.99	1.48	1.97
.29	3 1/2	.53	.79	1.04	1.56	2.08
.30	3 5/8	.56	.83	1.10	1.64	2.19
.31	3 3/4	.59	.87	1.15	1.73	2.30
.32	3 13/16	.61	.91	1.21	1.81	2.41
.33	3 15/16	.64	.95	1.27	1.89	2.52
.34	4 1/16	.67	1.00	1.32	1.98	2.64
.35	4 3/16	.70	1.04	1.38	2.07	2.75
.36	4 5/16	.73	1.09	1.44	2.16	2.87
.37	4 7/16	.77	1.13	1.50	2.25	2.99
.38	4 9/16	.80	1.18	1.57	2.34	3.11
.39	4 11/16	.83	1.23	1.63	2.43	3.24
.40	4 13/16	.87	1.28	1.69	2.53	3.36
.41	4 15/16	.90	1.32	1.76	2.62	3.49
.42	5 1/16	.93	1.37	1.82	2.72	3.61
.43	5 3/16	.97	1.42	1.89	2.81	3.74
.44	5 1/4	1.00	1.47	1.95	2.91	3.87

[1] Computed by the formula $Q = 3.247\ LH^{1.48} - \left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9} + 0.609H^{2.5}$

TABLE VI.—*Discharges (in cubic feet per second) through Cipolletti weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.45	5 3/8	1.04	1.53	2.02	3.01	4.01
.46	5 1/2	1.07	1.58	2.09	3.11	4.14
.47	5 5/8	1.11	1.63	2.16	3.21	4.28
.48	5 3/4	1.15	1.68	2.23	3.32	4.41
.49	5 7/8	1.18	1.74	2.30	3.42	4.55
.50	6	1.22	1.79	2.37	3.53	4.69
.51	6 1/8	1.26	1.85	2.44	3.64	4.83
.52	6 1/4	1.30	1.90	2.51	3.74	4.97
.53	6 3/8	1.34	1.96	2.59	3.85	5.12
.54	6 1/2	1.38	2.02	2.66	3.96	5.26
.55	6 5/8	1.42	2.07	2.74	4.07	5.41
.56	6 3/4	1.46	2.13	2.81	4.18	5.56
.57	6 13/16	1.50	2.19	2.89	4.30	5.71
.58	6 15/16	1.54	2.25	2.97	4.41	5.86
.59	7 1/16	1.58	2.31	3.05	4.53	6.01
.60	7 3/16	1.62	2.37	3.13	4.64	6.17
.61	7 5/16	1.67	2.43	3.20	4.76	6.32
.62	7 7/16	1.71	2.49	3.28	4.88	6.47
.63	7 9/16	1.75	2.55	3.37	5.00	6.63
.64	7 11/16	1.80	2.62	3.45	5.12	6.79
.65	7 13/16	1.84	2.68	3.53	5.24	6.95
.66	7 15/16	1.89	2.75	3.61	5.36	7.11
.67	8 1/16	1.93	2.81	3.70	5.48	7.28
.68	8 3/16	1.98	2.87	3.79	5.61	7.44
.69	8 1/4	2.02	2.94	3.87	5.73	7.61
.70	8 3/8	2.07	3.01	3.95	5.86	7.77
.71	8 1/2	2.12	3.07	4.04	5.98	7.94
.72	8 5/8	2.16	3.14	4.13	6.11	8.11
.73	8 3/4	2.21	3.21	4.22	6.24	8.28
.74	8 7/8	2.26	3.28	4.31	6.38	8.45
.75	9	2.31	3.35	4.40	6.51	8.62
.76	9 1/8	2.36	3.42	4.49	6.64	8.80
.77	9 1/4	2.41	3.49	4.58	6.77	8.97
.78	9 3/8	2.46	3.56	4.67	6.90	9.15
.79	9 1/2	2.51	3.63	4.76	7.04	9.33
.80	9 5/8	2.56	3.70	4.85	7.18	9.51
.81	9 3/4	2.61	3.77	4.95	7.31	9.69
.82	9 13/16	2.66	3.84	5.04	7.45	9.87
.83	9 15/16	2.71	3.92	5.14	7.59	10.05
.84	10 1/16	2.77	3.99	5.23	7.73	10.23
.85	10 3/16	2.82	4.07	5.33	7.87	10.42
.86	10 5/16	2.87	4.14	5.43	8.01	10.60
.87	10 7/16	2.93	4.22	5.52	8.15	10.79
.88	10 9/16	2.98	4.29	5.62	8.30	10.98
.89	10 11/16	3.04	4.37	5.72	8.44	11.17

TABLE VI.—*Discharges (in cubic feet per second) through Cipolletti weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
0.90	10 13/16	3.09	4.45	5.82	8.59	11.36
.91	10 15/16	3.15	4.53	5.92	8.73	11.55
.92	11 1/16	3.20	4.60	6.02	8.88	11.74
.93	11 3/16	3.26	4.68	6.13	9.03	11.94
.94	11 1/4	3.32	4.76	6.23	9.17	12.13
.95	11 3/8	3.37	4.84	6.33	9.32	12.33
.96	11 1/2	3.43	4.92	6.44	9.47	12.53
.97	11 5/8	3.49	5.00	6.55	9.62	12.72
.98	11 3/4	3.55	5.09	6.64	9.78	12.92
.99	11 7/8	3.61	5.17	6.75	9.93	13.12
1.00	12	3.67	5.25	6.86	10.08	13.32
1.01	12 1/8	.....	5.33	6.96	10.24	13.53
1.02	12 1/4	.....	5.42	7.07	10.40	13.73
1.03	12 3/8	.....	5.50	7.18	10.55	13.94
1.04	12 1/2	.....	5.59	7.29	10.71	14.15
1.05	12 5/8	.....	5.67	7.40	10.87	14.35
1.06	12 3/4	.....	5.76	7.51	11.03	14.56
1.07	12 13/16	.....	5.84	7.62	11.19	14.77
1.08	12 15/16	.....	5.93	7.73	11.35	14.98
1.09	13 1/16	.....	6.02	7.84	11.51	15.19
1.10	13 3/16	.....	6.11	7.96	11.68	15.41
1.11	13 5/16	.....	6.20	8.07	11.84	15.62
1.12	13 7/16	.....	6.29	8.18	12.00	15.83
1.13	13 9/16	.....	6.38	8.29	12.16	16.04
1.14	13 11/16	.....	6.47	8.41	12.33	16.26
1.15	13 13/16	.....	6.56	8.53	12.50	16.48
1.16	13 15/16	.....	6.65	8.65	12.67	16.70
1.17	14 1/16	.....	6.74	8.76	12.84	16.93
1.18	14 3/16	.....	6.83	8.88	13.01	17.15
1.19	14 1/4	.....	6.93	9.00	13.18	17.37
1.20	14 3/8	.....	7.02	9.12	13.35	17.59
1.21	14 1/2	.....	7.11	9.24	13.52	17.81
1.22	14 5/8	.....	7.20	9.36	13.69	18.03
1.23	14 3/4	.....	7.30	9.48	13.87	18.26
1.24	14 7/8	.....	7.40	9.60	14.04	18.49
1.25	15	.....	7.49	9.72	14.21	18.71
1.26	15 1/8	.....	.....	.....	14.39	18.94
1.27	15 1/4	.....	.....	.....	14.56	19.17
1.28	15 3/8	.....	.....	.....	14.74	19.41
1.29	15 1/2	.....	.....	.....	14.92	19.65
1.30	15 5/8	.....	.....	.....	15.11	19.88
1.31	15 3/4	.....	.....	.....	15.29	20.12
1.32	15 13/16	.....	.....	.....	15.46	20.35
1.33	15 15/16	.....	.....	.....	15.64	20.58
1.34	16 1/16	.....	.....	.....	15.82	20.82

TABLE VI.—*Discharges (in cubic feet per second) through Cipolletti weir notches*—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	*Inches.*					
1.35	16 3/16	.....	.....	.....	16.01	21.06
1.36	16 5/16	.....	.....	.....	16.19	21.29
1.37	16 7/16	.....	.....	.....	16.37	21.53
1.38	16 9/16	.....	.....	.....	16.56	21.78
1.39	16 11/16	.....	.....	.....	16.75	22.02
1.40	16 13/16	.....	.....	.....	16.94	22.27
1.41	16 15/16	.....	.....	.....	17.13	22.51
1.42	17 1/16	.....	.....	.....	17.32	22.75
1.43	17 3/16	.....	.....	.....	17.51	23.00
1.44	17 1/4	.....	.....	.....	17.70	23.25
1.45	17 3/8	.....	.....	.....	17.89	23.50
1.46	17 1/2	.....	.....	.....	18.08	23.75
1.47	17 5/8	.....	.....	.....	18.28	24.00
1.48	17 3/4	.....	.....	.....	18.47	24.25
1.49	17 7/8	.....	.....	.....	18.66	24.50
1.50	18	.....	.....	.....	18.85	24.75

The discharges through the Cipolletti notch, having a nominal crest length of 0.5 foot, did not follow the same law as those through the longer notches, possibly for the reasons noted on page 1067 for the 0.5-foot rectangular notch, and the use of such notches should be discouraged in favor of the 90° triangular notch, which measures small discharges more accurately.

The following formula represents the flow through the 0.5-foot Cipolletti notch, but is stated here only for technical reasons:

$$Q = 1.593H^{1.526}\left(1 + \frac{1}{800H^{2.3}}\right)0.587H^{2.53}$$

COMPARISON OF THE CIPOLLETTI FORMULA AND THE NEW FORMULA

The discharge values computed by the Cipolletti and new formulas are shown in graphic form in figure 10 and in tabular form in Table VII.

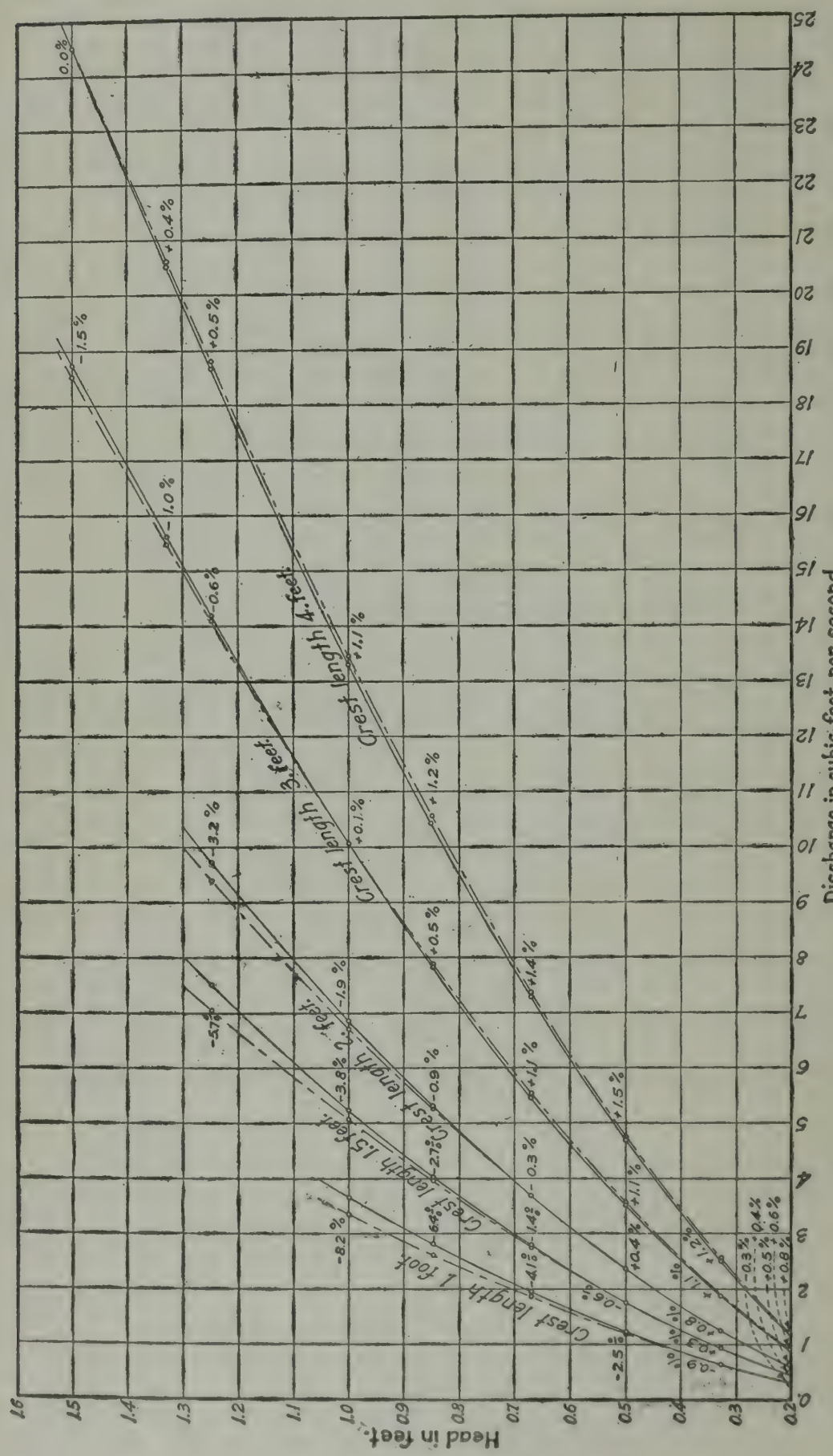

FIG. 10.—Curves showing discharges through Cipolletti weir notches of different lengths.

TABLE VII.—*Comparison of discharges through trapezoidal notches with side slopes of 1:4 computed by the Cipolletti formula and by the new formula.*[1]

Head.	1-foot crest.			1½-foot crest.			2-foot crest.			3-foot crest.			4-foot crest.		
	Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.	
		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.
Feet.															
0.20	0.302	0.301	99.7	0.450	0.452	100.4	0.599	0.602	100.5	0.898	0.903	100.6	1.20	1.21	100.8
.33	.644	.638	99.1	.954	.957	100.3	1.27	1.28	100.8	1.89	1.91	101.1	2.52	2.55	101.2
.50	1.22	1.19	97.5	1.79	1.78	99.4	2.37	2.38	100.4	3.53	3.57	101.1	4.69	4.76	101.5
.67	1.93	1.85	95.9	2.81	2.77	98.6	3.70	3.69	99.7	5.48	5.54	101.1	7.28	7.38	101.4
.85	2.82	2.64	93.6	4.07	3.96	97.3	5.33	5.28	99.1	7.87	7.91	100.5	10.42	10.55	101.2
1.00	3.67	3.37	91.8	5.25	5.05	96.2	6.86	6.73	98.1	10.09	10.10	100.1	13.33	13.47	101.1
1.25	.....	.....	.....	7.49	7.06	94.3	9.72	9.41	96.8	14.21	14.12	99.4	18.72	18.82	100.5
1.33	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.64	15.49	99.0	20.58	20.66	100.4
1.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	18.85	18.56	98.5	24.75	24.74	100.0

[1] Cipolletti formula: $Q = 3.367 LH^{3/2}$

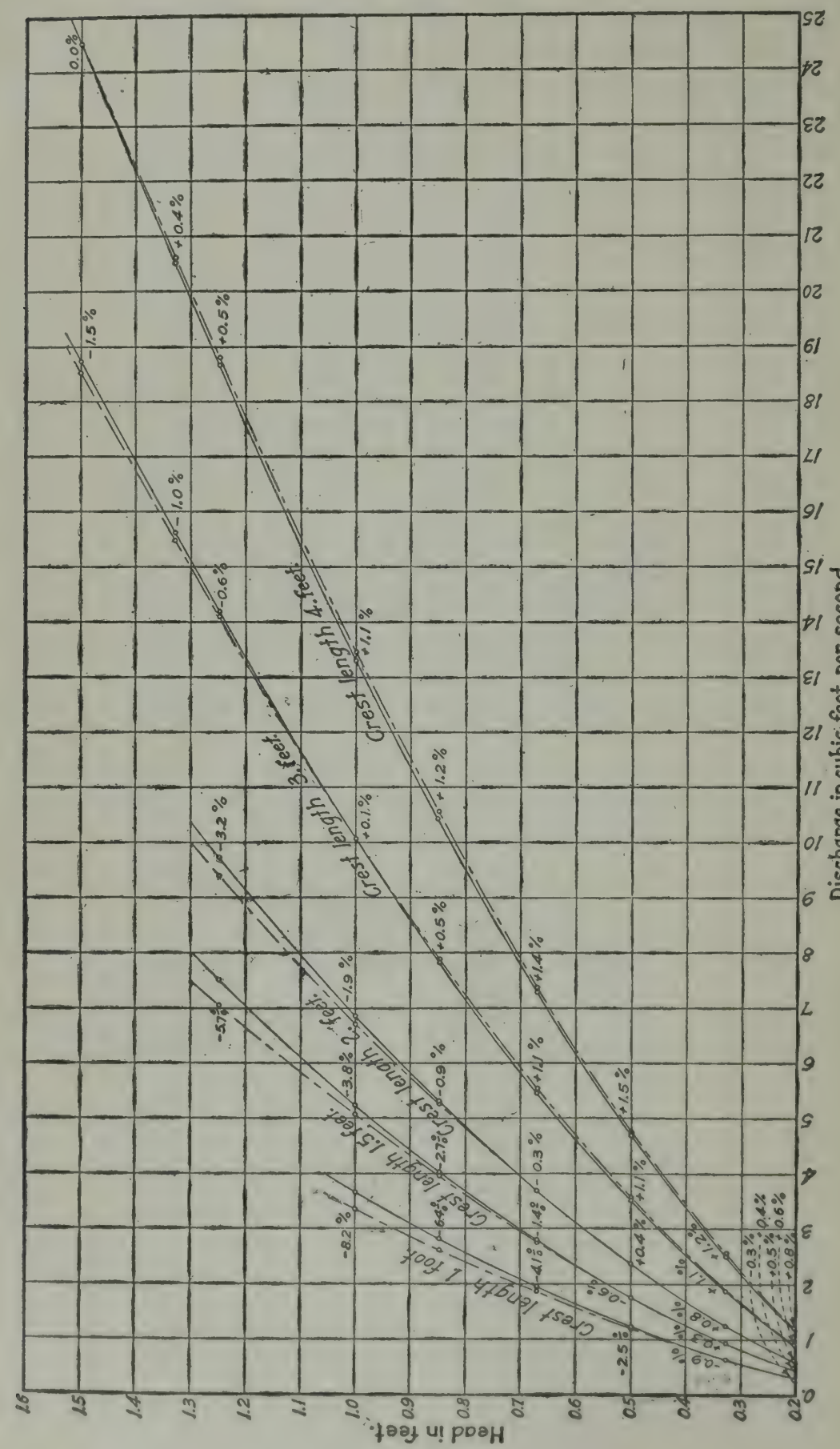

FIG. 10.—Curves showing discharges through Cipolletti weir notches of different lengths.

TABLE VII.—*Comparison of discharges through trapezoidal notches with side slopes of 1:4 computed by the Cipolletti formula and by the new formula.*[1]

Head.	1-foot crest.			1½-foot crest.			2-foot crest.			3-foot crest.			4-foot crest.		
	Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.		Discharge computed by new formula (cubic feet per second).	Discharge computed by Cipolletti formula.	
		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.
Feet.															
0.20	0.302	0.301	99.7	0.450	0.452	100.4	0.599	0.602	100.5	0.898	0.903	100.6	1.20	1.21	100.8
.33	.644	.638	99.1	.954	.957	100.3	1.27	1.28	100.8	1.89	1.91	101.1	2.52	2.55	101.2
.50	1.22	1.19	97.5	1.79	1.78	99.4	2.37	2.38	100.4	3.53	3.57	101.1	4.69	4.76	101.5
.67	1.93	1.85	95.9	2.81	2.77	98.6	3.70	3.69	99.7	5.48	5.54	101.1	7.28	7.38	101.4
.85	2.82	2.64	93.6	4.07	3.96	97.3	5.33	5.28	99.1	7.87	7.91	100.5	10.42	10.55	101.2
1.00	3.67	3.37	91.8	5.25	5.05	96.2	6.86	6.73	98.1	10.09	10.10	100.1	13.33	13.47	101.1
1.25	.....	.....	.....	7.49	7.06	94.3	9.72	9.41	96.8	14.21	14.12	99.4	18.72	18.82	100.5
1.33	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.64	15.49	99.0	20.58	20.66	100.4
1.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	18.85	18.56	98.5	24.75	24.74	100.0

[1] Cipolletti formula: $Q = 3.367LH^{3/2}$

The curves and the table show that with heads less than one-third the length of the crest the Cipolletti formula gives discharge values within 1.5 per cent of the actual discharges, therefore being somewhat more accurate than the Francis formula. The new formula, like the new formula for the rectangular weir, is not only more nearly accurate than the old formula, but also permits the use of heads greater than one-third the crest length. The maximum limit of the ratio of the head to the crest length was not ascertained, but the parts of the curves for the higher heads are consistent, there being no sudden breaks or changes of direction.

The new formula is more complicated than the Cipolletti formula, but because of its greater degree of accuracy it should be used in computing tables. The Cipolletti formula, however, is sufficiently accurate for field computations where only approximate discharge values are required.

Cipolletti notches do not give discharges proportional to the lengths of the crest, as has been commonly claimed, and consequently notches of this type have no advantages over rectangular notches (see p. 1098).

Formula Based on the Straight-Line Formula for Rectangular Notches

The difference between the discharges computed by the new rectangular-notch formula and the discharges taken from the curves plotted from the experimental data for the Cipolletti notches were determined for each 0.1 foot of head for the several lengths of notches. These values were then plotted logarithmically against the heads, and the equation of the average straight line representing the difference in discharge was found to be $D=.6H^{2.6}$. By adding the term $0.6H^{2.6}$ to the general formula for discharges through rectangular notches (p. 1071), the general formula for discharges through Cipolletti notches was found to be

$$Q=3.08L^{1.022}H^{(1.46+0.003L)}+0.6H^{2.6}$$

This formula gives discharge values that agree within a maximum of 1 per cent of the values indicated on the curves plotted from the experimental data, but the agreement is within 0.5 per cent for all but a very few points.

Table VIII gives the discharges through the notches used, computed by the two formulas deduced for the Cipolletti notches, and the discharge values indicated on the curves plotted from the experimental data.

TABLE VIII.—*Discharges (in cubic feet per second) for Cipolletti weir notches as shown by curves plotted from experimental data, and discharges computed by formulas on pages 1074 and 1080*

Head.	1.0050-foot notch.			1.5028-foot notch.			2.0002-foot notch.			3.0011-foot notch.			4.0058-foot notch.		
	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.
Feet.															
0.2	0.300	0.302	0.303	0.455	0.450	0.451	0.600	0.600	0.602	0.902	0.900	0.898	1.206	1.20	1.199
.3	.555	.563	.558	.829	.83	.827	1.109	1.10	1.100	1.647	1.64	1.639	2.193	2.19	2.188
.4	.866	.874	.866	1.280	1.28	1.275	1.694	1.69	1.694	2.535	2.53	2.519	3.360	3.36	3.357
.5	1.218	1.23	1.222	1.798	1.80	1.791	2.375	2.37	2.370	3.530	3.53	3.515	4.705	4.70	4.684
.6	1.622	1.63	1.626	2.370	2.37	2.369	3.141	3.13	3.125	4.650	4.64	4.624	6.179	6.18	5.156
.7	2.075	2.08	2.077	3.004	3.02	3.009	3.953	3.95	3.958	5.870	5.86	5.839	7.800	7.78	7.763
.8	2.565	2.57	2.571	3.706	3.71	3.704	4.845	4.85	4.859	7.185	7.18	7.150	9.537	9.52	9.492
.9	3.111	3.11	3.111	4.462	4.46	4.458	5.815	5.82	5.831	8.576	8.59	8.557	11.392	11.38	11.348
1.0	3.695	3.69	3.697	5.261	5.26	5.270	6.845	6.86	6.873	10.078	10.08	10.057	13.376	13.34	13.320
1.1	.....	.....	.....	6.137	6.12	6.138	7.941	7.96	7.983	11.655	11.68	11.647	15.425	15.43	15.404
1.2	.....	.....	.....	7.060	7.03	7.063	9.110	9.12	9.159	13.359	13.36	13.325	.....	.....	.....

The differences between the discharges through the 0.5-foot Cipolletti notch obtained from the curves plotted from the experimental data and the discharges computed by the formula for the 0.5-foot rectangular notch were determined and plotted logarithmically against the heads. The straight line representing these differences has the equation $D=0.56H^{2.55}$. By adding the term $0.56H^{2.55}$ to the formula for the discharge through the 0.5-foot rectangular notch, the formula for the discharge through a 0.5-foot Cipolletti notch becomes

$$Q=1.566H^{1.504}+0.56H^{2.55}$$

NOTCHES WITH SIDE SLOPES OF 1 TO 3 AND 1 TO 6

Experiments were made with notches having crest lengths of 2 feet and side slopes of 1 to 3 and 1 to 6, respectively. Since notches of only one length were used in each set of experiments, no general equations were deduced for notches of these types. The discharges obtained in the experiments for heads over 0.4 foot are shown graphically in figure 11. Discharges with heads less than 0.4 foot are approximately the same as those given in Tables II and VI.

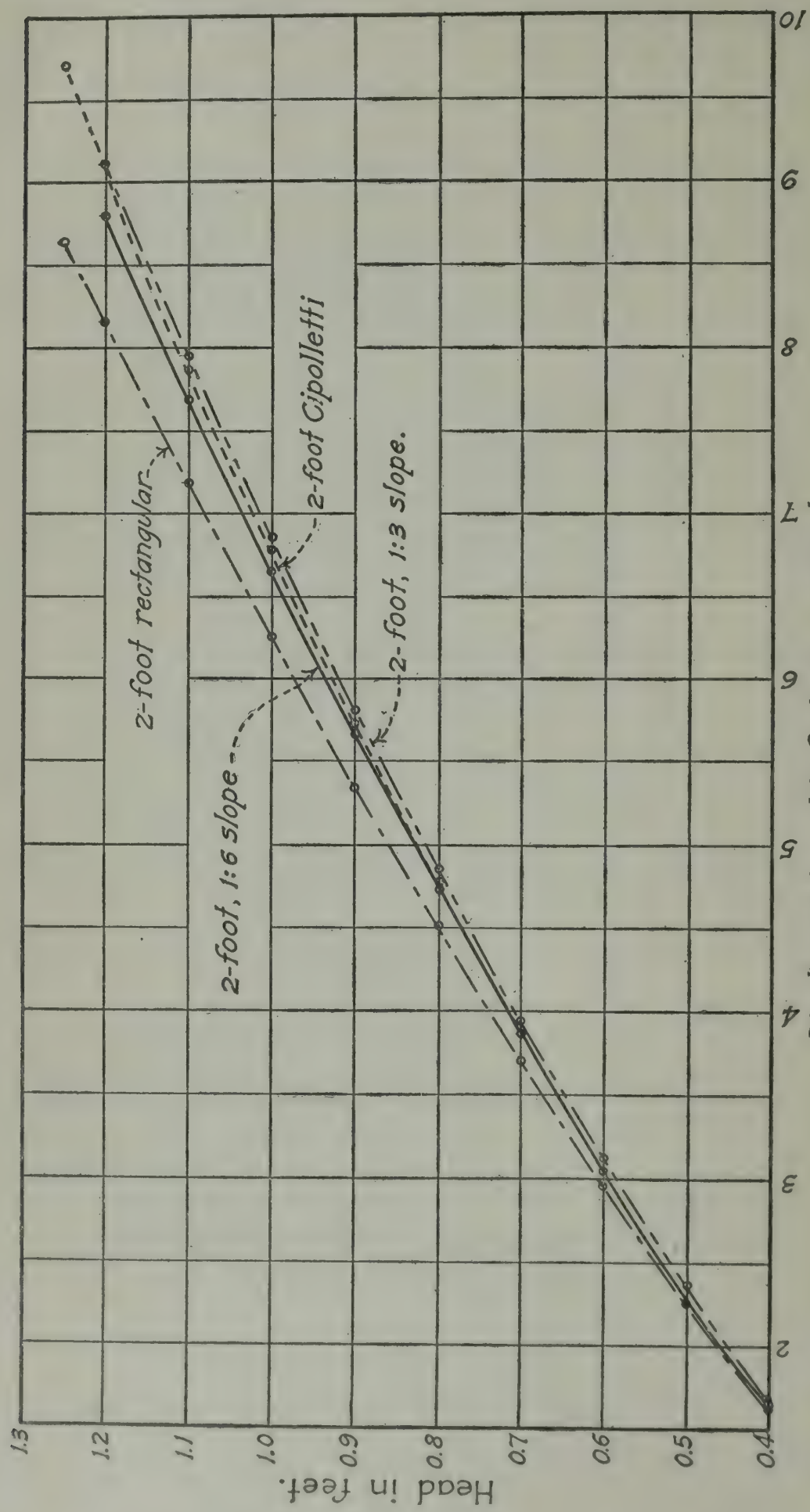

FIG. 11.—Curves showing discharges through 2-foot rectangular and Cipolletti notches and 2-foot notches having 1 to 3 and 1 to 6 side slopes.

TRIANGULAR NOTCHES

General theoretical formulas have been given for triangular notches (7, p. 46; 8, p. 168), and experiments with a 90° notch have been made by Thomson[1] (12, p. 181; 13, p. 154) and Barr.[2] In the Fort Collins laboratory 98 tests were made with heads ranging from 0.2 foot to 1.35 feet on weirs having triangular notches of 120°, 90°, 60°, 30° and approximately 28° 4′. The side slopes for the last-named notch are 1 horizontal to 4 vertical, and the tests were made with the idea that they might be of use in deriving a formula for discharges through Cipolletti notches.

DERIVATION OF FORMULAS

The discharges through the different notches when plotted logarithmically gave straight lines, as shown in figure 12. The equations for these lines were found to be as shown in Table IX.

TABLE IX.—*Equations for straight lines representing discharges through triangular notches*

Notch angle.	Slope of sides, horizontal vertical.	Equation of line.
120°	1.732	$Q=4.400H^{2.4870}$
90°	1.000	$Q=2.487H^{2.4805}$
60°	.577	$Q=1.446H^{2.4705}$
30°	.268	$Q=0.6848H^{2.4476}$
28°4′[a]	.250	$Q=0.6405H^{2.4448}$

[a] Approximate.

The discharging streams had a free fall in all the tests except those for the 120° notch. The upper portion of the stream over the 120° notch adhered to the edge of the notch for a distance of approximately 0.1 foot, the distance being quite uniform for all heads. The sides and crest of the notch used were of brass one-fourth inch thick, and were dressed at an angle of about 45° to a thickness of about one thirty-second inch at the edge. As the amount of adherence of nappe for the 120° notch depends upon the thickness of the edges of the notch, the use of such a notch is impracticable.

The data for the 120° notch having been excluded, the general formula for the discharge through the triangular notches of 28° 4′ to 90° was found to be

$$Q=(0.025+2.462\,S)\,H^{\left(2.5-\frac{0.0195}{S^{0.76}}\right)}$$

[1] The formula derived by Thomson for the 90° notch was $Q=0.305H^{3/2}$, in which Q is in cubic feet per minute and H is in inches.

[2] Barr found that with heads of 2 to 10 inches the coefficient C in Thomson's formula ($Q=CH^{5/2}$) varied from .3104 to .2995. Strickland found that Barr's coefficient C for any head could be computed from the formula $C=0.2907+\frac{0.028}{\sqrt{h}}$, h being in inches.

in which Q is the discharge in cubic feet per second, S is the slope of the sides, expressed decimally, and H is the head in feet.

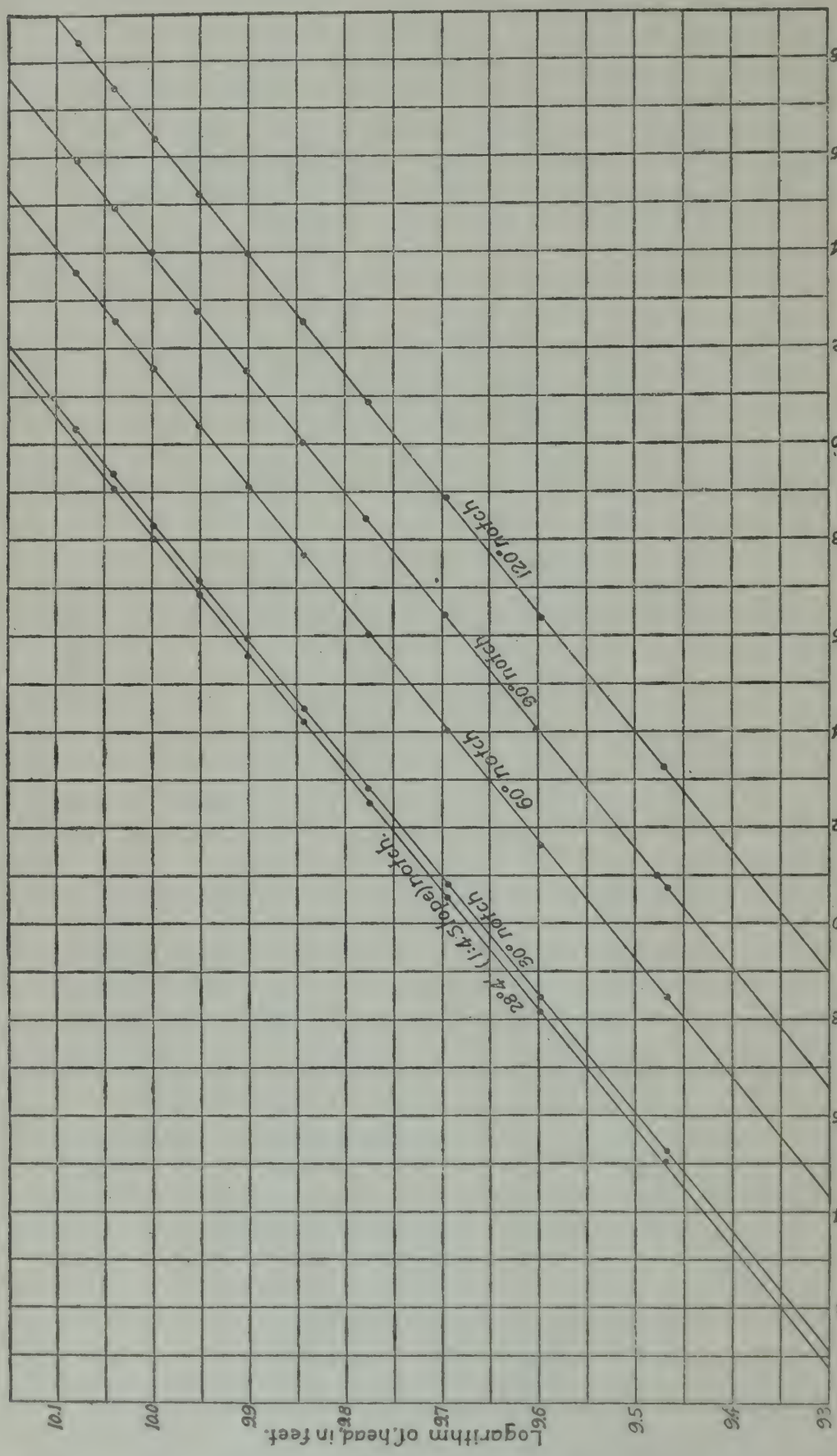

FIG. 12.—Logarithmic diagram of discharges through 28° 4′, 30°, 60°, 90°, and 120° triangular notches.

No experiments were made with notches between 90° and 120°, but a study of the working of the 120° notch led to the conclusion that the

application of the general formula given above can be extended to notches having side slopes of 1 to 1.4 (109° approximately).

Table X, computed by the new general formula, gives the discharges through notches of different shapes with heads up to 1.25 feet.

TABLE X.—*Discharges (in cubic feet per second) for triangular weir notches*[1]

Head.		Notch angle 28° 4′.	Notch angle 30°.	Notch angle 60°.	Notch angle 90°.
Feet.	*Inches.*				
0.20	2 3/8	0.012	0.013	0.027	0.046
.21	2 1/2	.014	.015	.031	.052
.22	2 5/8	.016	.017	.034	.058
.23	2 3/4	.018	.019	.038	.065
.24	2 7/8	.020	.021	.043	.072
.25	3	.022	.023	.047	.080
.26	3 1/8	.024	.025	.052	.088
.27	3 1/4	.026	.028	.057	.096
.28	3 3/8	.029	.030	.062	.105
.29	3 1/2	.031	.033	.068	.115
.30	3 5/8	.034	.036	.074	.125
.31	3 3/4	.037	.039	.080	.136
.32	3 13/16	.040	.042	.087	.147
.33	3 15/16	.043	.045	.094	.159
.34	4 1/16	.046	.049	.101	.171
.35	4 3/16	.049	.052	.108	.184
.36	4 5/16	.053	.056	.116	.197
.37	4 7/16	.056	.060	.124	.211
.38	4 9/16	.060	.064	.132	.225
.39	4 11/16	.064	.068	.141	.240
.40	4 13/16	.068	.073	.150	.256
.41	4 15/16	.072	.077	.160	.272
.42	5 1/16	.077	.082	.170	.289
.43	5 3/16	.081	.087	.180	.306
.44	5 1/4	.086	.092	.190	.324
.45	5 3/8	.091	.097	.201	.343
.46	5 1/2	.096	.102	.212	.362
.47	5 5/8	.101	.108	.224	.382
.48	5 3/4	.106	.114	.236	.403
.49	5 7/8	.112	.120	.248	.424
.50	6	.118	.126	.261	.445
.51	6 1/8	.123	.132	.274	.468
.52	6 1/4	.129	.138	.287	.491
.53	6 3/8	.136	.145	.301	.515
.54	6 1/2	.142	.152	.315	.539
.55	6 5/8	.148	.159	.330	.564
.56	6 3/4	.155	.166	.345	.590
.57	6 13/16	.162	.173	.360	.617
.58	6 15/16	.169	.181	.376	.644
.59	7 1/16	.176	.188	.392	.672

[1] Computed by the formula $Q=(0.025+2.462S)H\left(2.5-\frac{0.0195}{S^{0.75}}\right)$

TABLE X.—*Discharges (in cubic feet per second) for triangular weir notches*—Continued

Head.		Notch angle 28° 4′.	Notch angle 30°.	Notch angle 60°.	Notch angle 90°.
Feet.	*Inches.*				
0.60	7 3/16	0.184	0.196	0.409	0.700
.61	7 5/16	.191	.204	.426	.730
.62	7 7/16	.199	.212	.444	.760
.63	7 9/16	.207	.221	.462	.790
.64	7 11/16	.215	.230	.480	.822
.65	7 13/16	.223	.239	.499	.854
.66	7 15/16	.232	.248	.518	.887
.67	8 1/16	.241	.257	.537	.921
.68	8 3/16	.250	.266	.557	.955
.69	8 1/4	.259	.276	.578	.991
.70	8 3/8	.268	.286	.599	1.03
.71	8 1/2	.277	.296	.620	1.06
.72	8 5/8	.287	.306	.642	1.10
.73	8 3/4	.297	.317	.664	1.14
.74	8 7/8	.307	.328	.687	1.18
.75	9	.317	.339	.710	1.22
.76	9 1/8	.327	.350	.734	1.26
.77	9 1/4	.338	.361	.758	1.30
.78	9 3/8	.349	.373	.782	1.34
.79	9 1/2	.360	.385	.807	1.39
.80	9 5/8	.371	.397	.833	1.43
.81	9 3/4	.383	.409	.859	1.48
.82	9 13/16	.394	.421	.885	1.52
.83	9 15/16	.406	.434	.912	1.57
.84	10 1/16	.418	.447	.940	1.61
.85	10 3/16	.430	.460	.968	1.66
.86	10 5/16	.443	.473	.996	1.71
.87	10 7/16	.456	.487	1.02	1.76
.88	10 9/16	.469	.501	1.05	1.81
.89	10 11/16	.482	.515	1.08	1.86
.90	10 13/16	.495	.529	1.11	1.92
.91	10 15/16	.509	.544	1.15	1.97
.92	11 1/16	.522	.558	1.18	2.02
.93	11 3/16	.536	.573	1.21	2.08
.94	11 1/4	.551	.589	1.24	2.13
.95	11 3/8	.565	.604	1.27	2.19
.96	11 1/2	.580	.620	1.31	2.25
.97	11 5/8	.595	.636	1.34	2.31
.98	11 3/4	.610	.652	1.38	2.37
.99	11 7/8	.625	.668	1.41	2.43
1.00	12	.641	.685	1.45	2.49
1.01	12 1/8	.656	.702	1.48	2.55
1.02	12 1/4	.672	.719	1.52	2.61
1.03	12 3/8	.688	.736	1.56	2.68
1.04	12 1/2	.705	.754	1.59	2.74
1.05	12 5/8	.722	.772	1.63	2.81
1.06	12 3/4	.739	.790	1.67	2.87
1.07	12 13/16	.756	.808	1.71	2.94
1.08	12 15/16	.773	.827	1.75	3.01
1.09	13 1/16	.791	.846	1.79	3.08

TABLE X.—*Discharges (in cubic feet per second) for triangular weir notches*—Con.

Head.		Notch angle 28° 4′.	Notch angle 30°.	Notch angle 60°.	Notch angle 90°.
Feet.	*Inches.*				
1. 10	13 3/16	0. 809	0. 865	1. 83	3. 15
1. 11	13 5/16	. 827	. 884	1. 87	3. 22
1. 12	13 7/16	. 845	. 904	1. 91	3. 30
1. 13	13 9/16	. 864	. 924	1. 96	3. 37
1. 14	13 11/16	. 882	. 944	2. 00	3. 44
1. 15	13 13/16	. 901	. 964	2. 04	3. 52
1. 16	13 15/16	. 921	. 985	2. 09	3. 59
1. 17	14 1/16	. 940	1. 01	2. 13	3. 67
1. 18	14 3/16	. 960	1. 03	2. 18	3. 75
1. 19	14 1/4	. 980	1. 05	2. 22	3. 83
1. 20	14 3/8	1. 00	1 07	2. 27	3. 91
1. 21	14 1/2	1. 02	1. 09	2. 32	3. 99
1. 22	14 5/8	1. 04	1. 11	2. 36	4. 07
1. 23	14 3/4	1. 06	1. 14	2. 41	4. 16
1. 24	14 7/8	1. 08	1. 16	2. 46	4. 24
1. 25	15	1. 11	1. 19	2. 51	4. 33

Although weirs with triangular notches are well suited to a comparatively wide range of discharges, they are especially well adapted for the measurement of small discharges and may be used to measure accurately quantities so small that they would not pass through trapezoidal or rectangular notches without adhering to the crests. The use of weirs with triangular notches requires slightly more fall than is required with trapezoidal or rectangular notches—that is, a head of 2 feet is required to deliver approximately 14 cubic feet per second through a 90° triangular notch, while the same discharge would be delivered through a 3-foot rectangular notch with a head of 1.31 feet, or through a 4-foot rectangular notch with a head of 1.07 feet.

Weirs with 90° notches are simpler in construction than any other type of weir and are the most practical type for small or medium-sized discharges. The approximate formula $Q = 2.49H^{2.48}$ gives discharge values for 90° notches, which agree very closely with the values obtained with the general formula.

Comparison of New Formula and Old Formula

The discharges for the 90° notch computed by the new and the old formulas are compared in Table XI:

Table XI.—*Comparison of new formula and old formula*

Head.	Discharge computed by new formula (cubic feet per second).	Discharge computed by old formula, $Q=2.53H^{5/2}$.	
		Discharge in cubic feet per second.	Percentage of discharge computed by new formula.
Feet.			
0.20	0.046	0.045	97.8
.33	.159	.158	99.4
.50	.445	.447	100.4
.67	.921	.930	101.0
.85	1.66	1.69	101.8
1.00	2.49	2.53	101.6
1.25	4.33	4.42	102.1

As no experiments have been made in the past to determine the coefficients in general formulas for notches of 28° 4′, 30°, or 60°, no comparison could be made with the discharges through such notches computed with the new formula.

CIRCULAR NOTCHES

Apparently no experiments have ever been made with circular or semicircular notches placed in a vertical position with heads less than the height of the opening. In order to throw light upon the probable discharges through such notches and obtain data to use in determining the flow through circular head gates when acting as weirs rather than as orifices, 50 tests were made with thin-edged circular notches, 17 being with a notch 0.4995 foot in diameter and 33 with a notch 1.0025 feet in diameter; and 34 tests were made with semicircular notches, 15 being with a notch 1.5011 feet in diameter and 19 with a notch 1.9990 feet in diameter. The discharge data obtained are shown graphically in figure 13.

CONDITIONS OF NOTCH EDGES REQUIRED TO INSURE FREE FLOW

The impression is common that the terms "thin edges" and "sharp crests," as applied to weir notches, mean knife edges. Such edges are not necessary, and the edges are sufficiently sharp or thin if the upstream corner of the notch edges is a distinct angle of 90° or less and the thickness of the notch edges is not so great that the water will adhere to them. The allowable thickness of the edges depends upon the head that is being used. Experiments made in the laboratory with notches having edges

¼ inch thick showed that while water would adhere to the notch edges

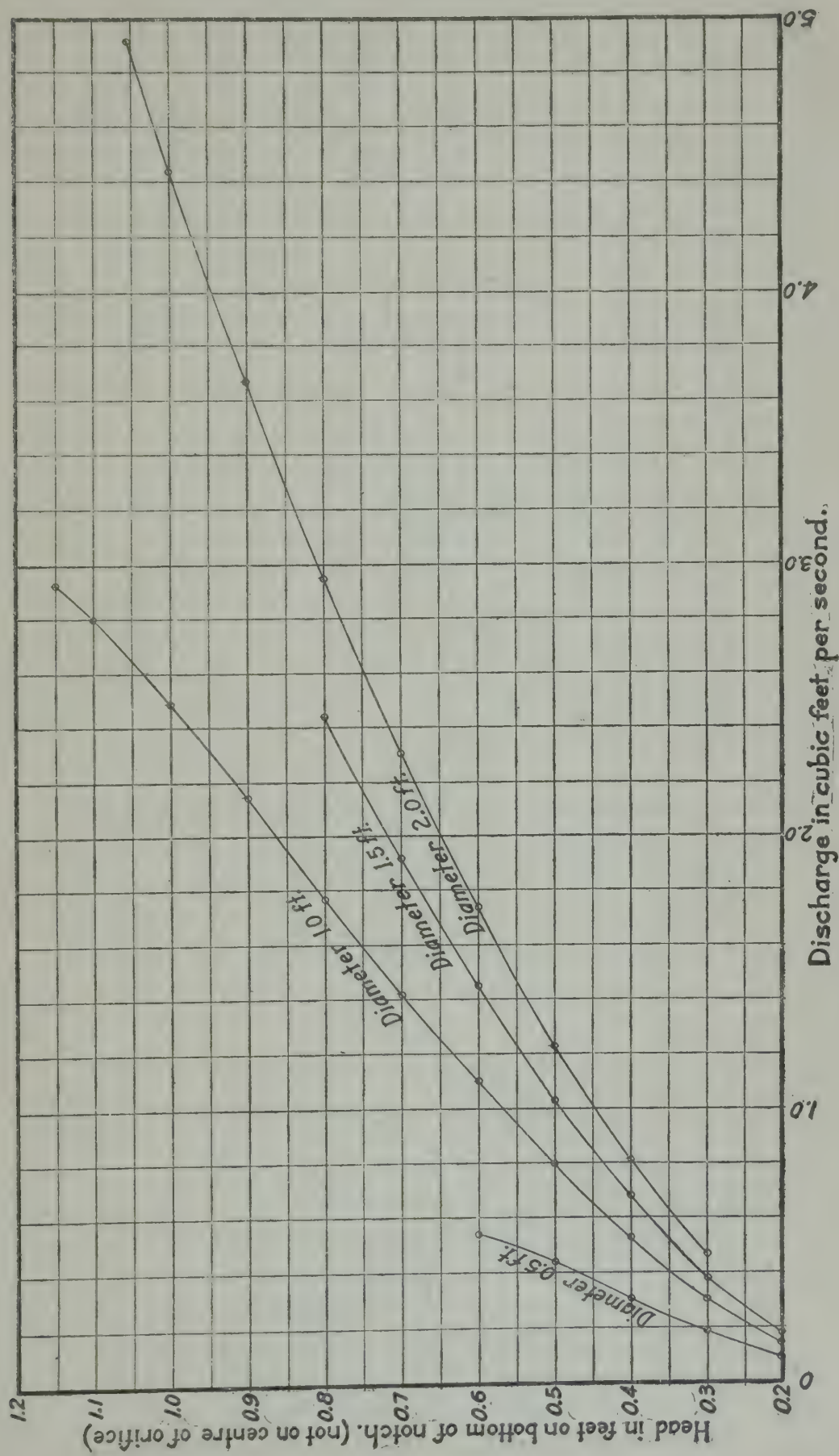

FIG. 13.—Curves showing discharges through circular weir notches.

with a head of 0.15 foot, there was no adherence with heads of 0.2 foot and over.

Notches with angles made as precisely as those used in the test would not be practicable for field use, and consequently a maximum thickness of 1/8 inch probably would be safer than 1/4 inch where heads as low as 0.2 foot will be used. While no experiments were made, edges as thick as 3/4 inch probably can be used where the minimum head will be 1 foot.

The edges of the weir notches must be straight, true, and rigid. These conditions are best insured by using angle irons or similar material that can be securely fastened to the bulkheads, as wood edges become splintered and warped, and thin sheet-metal weir plates buckle and bend easily. Regardless of the material used, the notches will be more permanent and reliable if the upstream corners of the notches are made definitely angular and the edges are left as thick as possible and still permit a free flow.

DISTANCE FROM NOTCH AT WHICH HEAD SHOULD BE MEASURED

In connection with the experiments with notches of different types, measurements were made to determine the transverse and longitudinal curves of the water surface upstream from the weirs when different heads were being used. These measurements showed that the extent of the curves backward from and to the sides of the notches depends upon the length of the crest and the head being used. Plots of the data obtained show that measurements of head should be made either at a distance of at least $4H$ upstream from the notch or at a distance of at least $2H$ sidewise from the end of the crest of the notch.

Table XII gives the errors and the percentage of error made in computing discharges for notches of different shapes and sizes with different heads caused by errors of 0.01 foot in reading the heads.

TABLE XII.—*Errors and percentage of error in computed discharges caused by 0.01-foot error in reading the heads*

RECTANGULAR WEIRS

Correct head.	Error.									
	1-foot crest.		1½-foot crest.		2-foot crest.		3-foot crest.		4-foot crest.	
Feet.	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*
0.20	0.021	7.22	0.033	7.52	0.044	7.48	0.067	7.55	0.09	7.56
.30	.026	4.94	.04	5.03	.05	4.67	.08	4.97	.10	4.63
.40	.029	3.61	.05	4.13	.06	3.68	.09	3.66	.12	3.64
.50	.04	3.60	.05	2.98	.07	3.10	.10	2.92	.14	3.06
.60	.04	2.76	.05	2.27	.07	2.36	.11	2.46	.14	2.33
.70	.04	2.20	.06	2.17	.07	1.89	.12	2.14	.16	2.13
.80	.04	1.81	.06	1.79	.08	1.77	.12	1.76	.17	1.86
.90	.05	1.91	.07	1.76	.09	1.68	.13	1.60	.18	1.65
1.00	.05	1.63	.07	1.51	.09	1.44	.14	1.48	.19	1.49
1.10	.....	.....	.07	1.31	.09	1.25	.14	1.28	.19	1.30
1.20	.....	.....	.07	1.16	.10	1.23	.15	1.21	.20	1.20
1.30	.....	.....	.....	.....	.....	.....	.16	1.15	.21	1.12
1.40	.....	.....	.....	.....	.....	.....	.16	1.03	.22	1.05
1.50	.....	.....	.....	.....	.....	.....	.16	.93	.23	1.00

TABLE XII.—*Errors and percentage of error in computed discharges caused by 0.01-foot error in reading the heads*—Continued

CIPOLLETTI WEIRS

Correct head.	Error.									
	1-foot crest.		1½-foot crest.		2-foot crest.		3-foot crest.		4-foot crest.	
Feet.	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*	*Cu. ft. per sec.*	*Per ct.*
0.20	0.022	7.3	0.034	7.6	0.045	7.5	0.068	7.6	0.09	7.5
.30	.028	5.0	.041	5.0	.055	5.0	.082	5.0	.11	5.0
.40	.034	3.9	.05	3.9	.07	4.1	.09	3.6	.12	3.6
.50	.04	3.3	.05	2.8	.07	3.0	.11	3.1	.14	3.0
.60	.04	2.5	.06	2.5	.08	2.6	.12	2.6	.15	2.4
.70	.05	2.4	.07	2.3	.09	2.3	.13	2.2	.17	2.2
.80	.05	2.0	.07	1.9	.09	1.9	.14	1.9	.18	1.9
.90	.05	1.6	.08	1.8	.10	1.7	.15	1.7	.19	1.7
1.00	.06	1.6	.08	1.5	.11	1.6	.15	1.5	.20	1.5
1.10	.....	.....	.09	1.5	.12	1.5	.17	1.5	.21	1.4
1.20	.....	.....	.09	1.3	.12	1.3	.17	1.3	.22	1.3
1.30	.....	.....	.....	.....	.....	.....	.18	1.2	.24	1.2
1.40	.....	.....	.....	.....	.....	.....	.19	1.1	.24	1.1

90° TRIANGULAR WEIRS

0.20	0.006	13.04	.....	.....	.....	.....	.....	.....	.....	.....
.50	.022	4.94	.....	.....	.....	.....	.....	.....	.....	.....
.70	.04	3.9	.....	.....	.....	.....	.....	.....	.....	.....
1.00	.06	2.4	.....	.....	.....	.....	.....	.....	.....	.....
1.25	.09	2.1	.....	.....	.....	.....	.....	.....	.....	.....

EFFECTS OF DIFFERENT END AND BOTTOM CONTRACTIONS UPON DISCHARGES

RECTANGULAR AND CIPOLLETTI NOTCHES

To determine the effect of different end and bottom contractions upon the discharges through rectangular and Cipolletti notches, 120 tests were made with 1-foot rectangular notches, 72 with 3-foot rectangular notches, 205 with 1-foot Cipolletti notches, and 89 with 3-foot Cipolletti notches. Heads of 0.2 foot, 0.6 foot, and 1 foot were used with each notch. The end contractions (the distances of the sides of the weir box from the ends of the crest) and the bottom contraction (the distance of the bottom of the weir box below the crest of the notch) for each notch were varied from 0.5 foot to 3 feet by increments of 0.5 foot. The discharges under the different conditions were compared with those obtained with the standard weir box. The small error in the experimental determinations of the discharges with a 0.2-foot head caused such large percentages of error in the discharges that they were unreliable and so were not included.

Figures 14 and 15 and Tables XIII and XIV show the percentages of increase in discharges and the velocities of approach with heads of 0.6 foot and 1 foot under the different conditions of contractions. The equations of the curve are all of the general form, $e = a(V + b)^n$, in which e

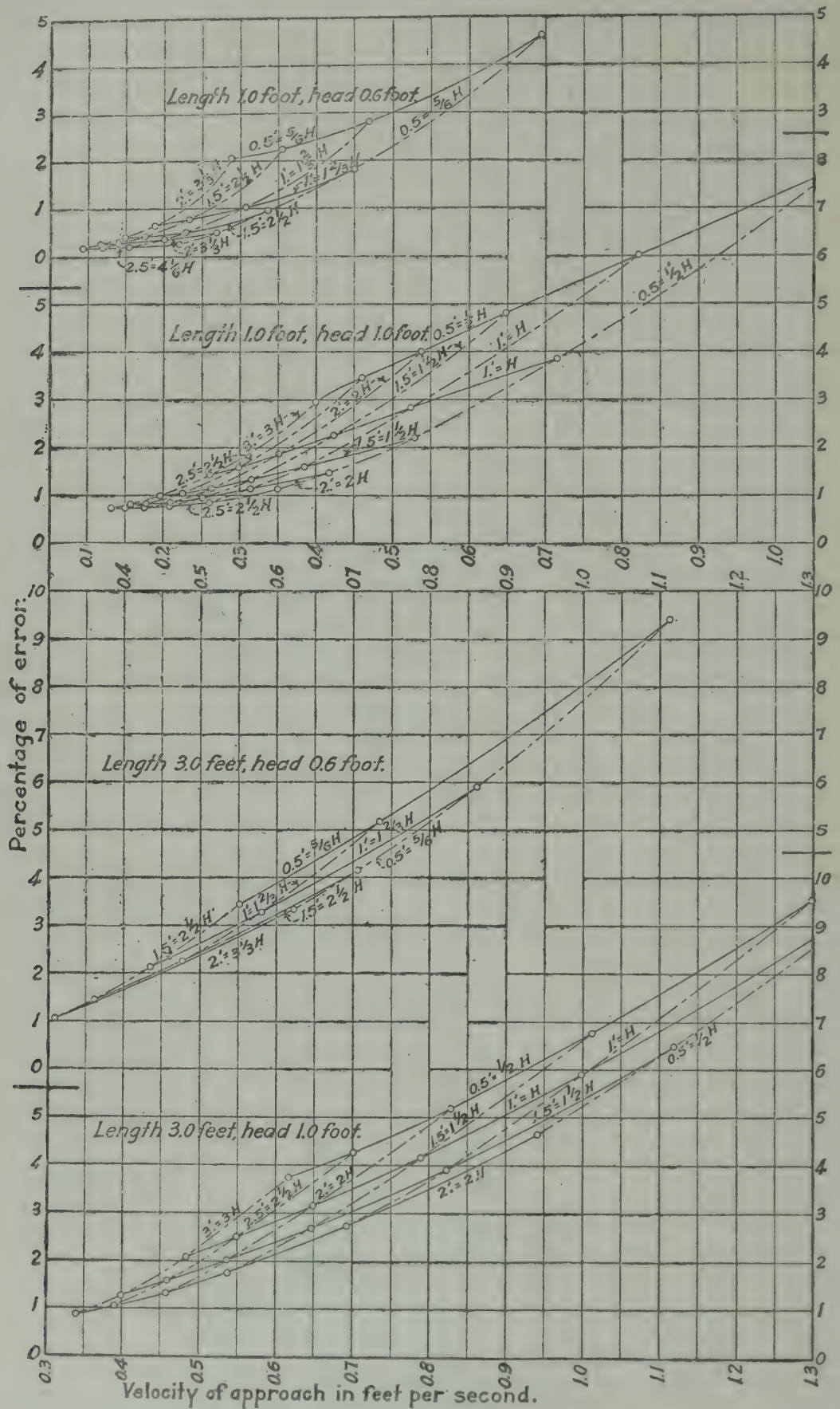
Length 1.0 foot, head 0.6 foot.
Length 1.0 foot, head 1.0 foot.
Length 3.0 feet, head 0.6 foot.
Length 3.0 feet, head 1.0 foot.
Percentage of error.
Velocity of approach in feet per second.

FIG. 14.—Curves showing effect of different end and bottom contractions upon discharges through 1-foot and 3-foot rectangular notches with heads of 0.6 and 1 foot. Full lines show end contractions; dot-dash lines show side contractions.

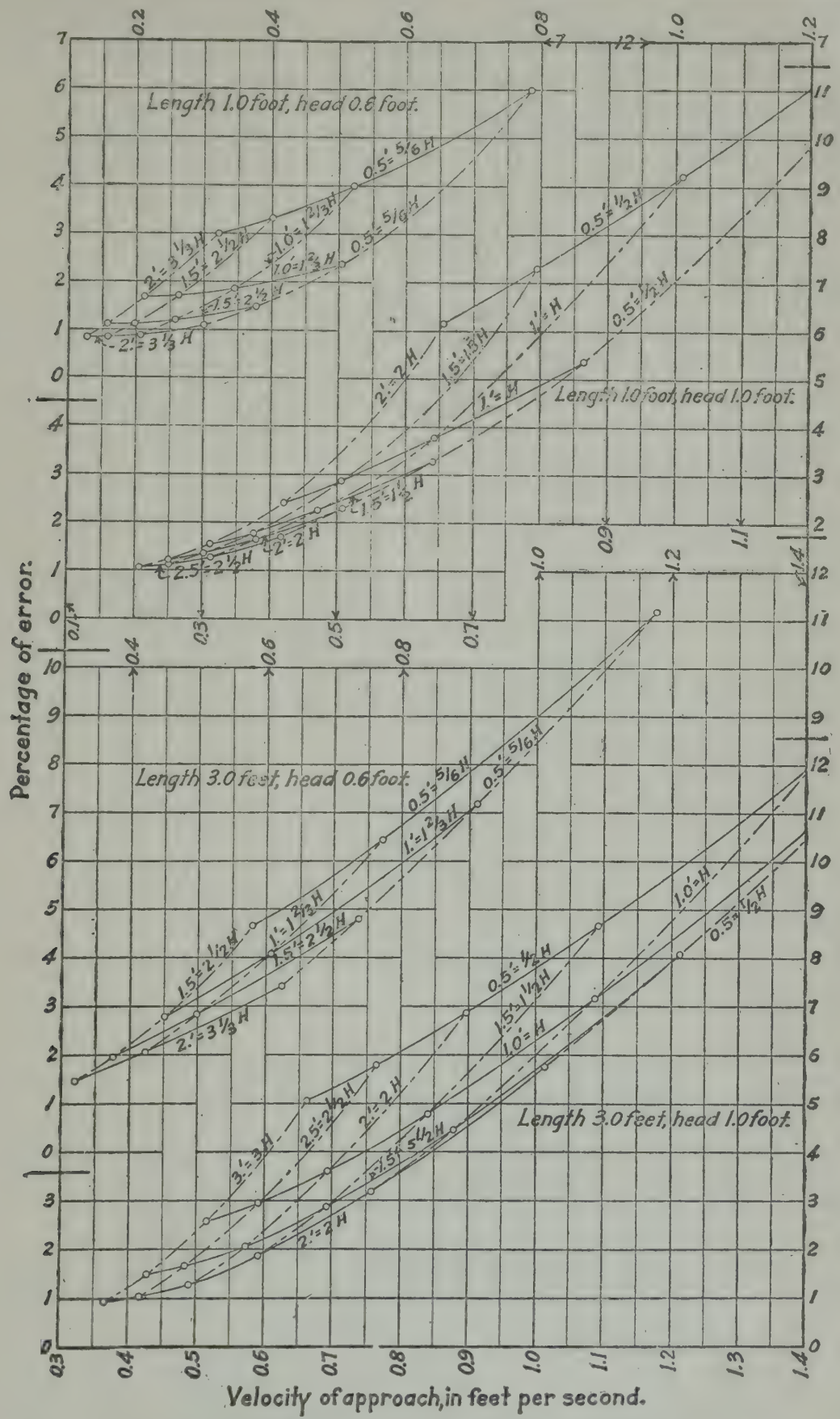
Length 1.0 foot, head 0.6 foot.
Length 1.0 foot, head 1.0 foot.
Length 3.0 feet, head 0.6 foot.
Length 3.0 feet, head 1.0 foot.
Percentage of error.
Velocity of approach, in feet per second.

FIG. 15.—Curves showing the effect of different end and bottom contractions upon the discharges through 1-foot and 3-foot Cipolletti weir notches with heads of 0.6 and 1 foot. Full lines show end contractions in feet; dot-dash lines show bottom contractions in feet.

is the percentage of increase in discharge, V is the average velocity of approach, and a, b, and n are constants for each size of each type of notch.

TABLE XIII.—*Velocities of approach (in feet per second) and percentages of increase in discharges through rectangular notches caused by different end and bottom contractions*

HEAD, 0.6 FOOT

Bottom contraction.	End contractions.	1-foot notch.		1½-foot notch.		2-foot notch.		3-foot notch.		4-foot notch.	
		Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.
Feet.	*Feet.*		*Per ct.*		*Per ct.*		*Per ct.*		*Per ct.*		*Per ct.*
	2.5	0.094	0.17	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.115	.26	.....	.....	.....	.....	.....	.....	.....	.....
2	1.5	.148	.39	.....	.....	.....	.....	.....	.....	.....	.....
	1.0	.188	.66	.....	.....	.....	.....	.....	.....	.....	.....
	.5	.288	2.05	.....	.....	.....	.....	.....	.....	.....	.....
	2.5	.119	.17	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.141	.30	0.191	0.53	0.239	0.74	0.308	1.07	0.365	1.33
1½	1.5	.175	.40	.234	.73	.286	1.01	.363	1.44	.416	1.74
	1.0	.234	.76	.304	1.24	.361	1.62	.435	2.12	.489	2.49
	.5	.355	2.26	.....	.....	.....	.....	.552	3.41	.....	.....
	2.5	.154	.19	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.209	.36	.....	.....	.....	.....	.....	.....	.....	.....
1	1.5	.229	.50	.311	1.09	.377	1.55	.478	2.25	.552	2.79
	1.0	.308	1.01	.400	1.77	.476	2.39	.577	3.22	.650	3.83
	.5	.469	2.84	.573	3.74	.646	4.38	.735	5.15	.794	5.64
	2.5	.221	.25	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.268	.50	.368	1.30	.460	2.05	.624	3.34	.711	4.05
½	1.5	.337	.94	.453	1.94	.555	2.84	.705	4.17	.818	5.15
	1.0	.450	1.84	.588	3.22	.704	4.35	.862	5.92	.975	7.01
	.5	.695	4.63	.852	6.43	.970	7.79	1.112	9.40	1.208	10.50

HEAD, 1 FOOT

	2.5	0.132	0.74	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.157	.81	0.213	0.82	0.269	0.84	0.342	0.83	0.402	0.87
3	1.5	.196	.99	.260	1.08	.317	1.14	.399	1.22	.460	1.29
	1.0	.260	1.40	.337	1.63	.398	1.81	.484	2.06	.543	2.22
	.5	.40	2.94	.477	3.22	.540	3.44	.616	3.72	.661	3.88
	2.5	.150	.74	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.178	.82	.242	.88	.302	.94	.391	1.04	.460	1.11
2½	1.5	.224	1.05	.297	1.21	.362	1.34	.461	1.57	.528	1.69
	1.0	.299	1.58	.385	1.89	.457	2.14	.553	2.50	.623	2.76
	.5	.462	3.42	.549	3.73	.625	3.99	.704	4.25	.760	4.48
	2.5	.175	.73	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.209	.84	.284	.97	.352	1.11	.458	1.30	.539	1.42
2	1.5	.261	1.13	.348	1.42	.424	1.67	.538	2.01	.620	2.28
	1.0	.353	1.83	.450	2.28	.535	2.63	.648	3.14	.733	3.52
	.5	.538	4.01	.646	4.46	.728	4.80	.829	5.17	.895	5.47
	2.5	.208	.74	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.252	.94	.341	1.18	.424	1.41	.539	1.71	.638	1.98
1½	1.5	.314	1.31	.418	1.74	.512	2.12	.648	2.65	.750	3.07
	1.0	.424	2.24	.544	2.87	.646	3.40	.790	4.14	.889	4.68
	.5	.648	4.80	.784	5.53	.885	6.09	1.013	6.77	1.091	7.20
	2.5	.260	.82	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.314	1.12	.427	1.57	.532	2.00	.694	2.69	.810	3.15
1	1.5	.385	1.59	.528	2.37	.645	2.99	.820	3.91	.952	4.60
	1.0	.525	2.83	.688	3.86	.825	4.73	.999	5.87	1.135	6.77
	.5	.822	6.00	.994	7.29	1.129	8.29	1.298	9.55	1.405	10.27
	2.5	.350	1.11	.....	.....	.....	.....	.....	.....	.....	.....
	2.0	.417	1.45	.575	2.40	.720	3.27	.943	4.62	1.120	5.65
½	1.5	.530	2.20	.710	3.53	.875	4.73	1.119	6.50	1.308	7.88
	1.0	.716	3.83	.930	5.65	1.118	7.23	1.380	9.40	1.576	11.2
	.5	1.120	8.25	1.37	11.0	1.58	13.3	1.83	16.01	2.01	18.0

TABLE XIV.—*Velocities of approach (in feet per second) and percentages of increase in discharges through Cipolletti notches caused by different bottom and end contractions*

HEAD, 0.6 FOOT

Bottom contraction.	End contractions.	1-foot notch.		1½-foot notch.		2-foot notch.		3-foot notch.		4-foot notch.	
		Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.
Feet.	*Feet.*		*Per ct.*		*Per ct.*		*Per ct.*		*Per ct.*		*Per ct.*
1½	2.0	0.158	0.84	0.207	1.02	0.251	1.21	0.321	1.45	0.373	1.61
	1.5	.196	1.11	.255	1.38	.304	1.60	.377	1.95	.429	2.19
	1.0	.260	1.70	.329	2.08	.381	2.36	.454	2.77	.504	3.02
	.5	.400	3.32	.469	3.83	.518	4.20	.580	4.66	.617	4.93
1	2.0	.205	.90	.274	1.25	.331	1.55	.425	2.05	.492	2.39
	1.5	.257	1.20	.335	1.71	.400	2.17	.500	2.82	.569	3.30
	1.0	.344	1.84	.434	2.60	.501	3.17	.607	4.06	.671	4.60
	.5	.529	4.00	.622	4.92	.690	5.61	.770	6.41	.826	6.98
½	2.0	.300	1.11	.399	1.81	.487	2.42	.625	3.40	.725	4.09
	1.5	.377	1.51	.492	2.55	.589	3.44	.737	4.79	.847	5.80
	1.0	.505	2.39	.636	3.93	.750	5.30	.908	7.18	1.013	8.39
	.5	.782	6.03	.932	8.02	1.037	9.43	1.173	11.28	1.263	12.48

HEAD, 1 FOOT

2	2.0	0.250	1.19	0.322	1.22	0.386	1.24	0.488	1.28	0.561	1.30
	1.5	.314	1.52	.397	1.70	.467	1.84	.575	2.08	.648	2.22
	1.0	.422	2.40	.514	2.80	.590	3.15	.698	3.62	.769	3.92
	.5	.655	6.16	.746	6.41	.813	6.61	.896	6.88	.951	7.01
1½	2.0	.300	1.34	.388	1.49	.465	1.61	.590	1.82	.680	1.98
	1.5	.378	1.78	.477	2.10	.562	2.40	.693	2.85	.785	3.17
	1.0	.508	2.89	.622	3.53	.714	4.06	.844	4.79	.937	5.31
	.5	.795	7.29	.906	7.79	.989	8.18	1.094	8.64	1.163	8.95
1	2.0	.374	1.60	.489	2.06	.586	2.44	.758	3.13	.864	3.55
	1.5	.471	2.20	.601	2.92	.710	3.55	.888	4.52	1.003	5.19
	1.0	.643	3.76	.787	4.83	.908	5.73	1.083	7.07	1.200	7.92
	.5	1.010	9.20	1.159	10.28	1.271	11.08	1.410	12.09	1.503	12.72
½	2.5	.64	3.3	.818	4.8	.968	6.07	1.21	8.07	1.391	9.56
	2.0	.508	2.30	.660	3.64	.799	4.87	1.013	6.73	1.202	8.39
	1.5	.640	3.30	.818	4.80	.969	6.09	1.210	8.08	1.391	9.56
	1.0	.864	5.40	1.077	7.58	1.258	9.43	1.505	11.95	1.688	13.81
	.5	1.390	11.89	1.605	14.63	1.782	16.85	2.015	19.80	.....	.....

Figure 16 shows the variation of the percentages of increase in the discharges through a 1-foot rectangular notch, with heads of 0.6 foot and 1 foot as the ratio of the cross-sectional area of the weir box (A) to the area of the weir notch (a), decreased with the use of different end and bottom contractions. From these curves it will be seen that changing the position of the sides of the weir box and leaving the bottom in a fixed position has a greater effect upon the discharges than leaving the sides fixed and moving the bottom. This indicates that end contractions have more effect upon the discharges than do bottom contractions. With end contractions equal to $2H$ and a bottom contraction equal to $3H$, or end contractions equal to $3H$ and a bottom contraction equal to $2H$, the mean velocities of approach are about one-third foot per second and the discharges with medium to high heads do not agree closer than approximately 1 per cent with the discharges computed by the formula.

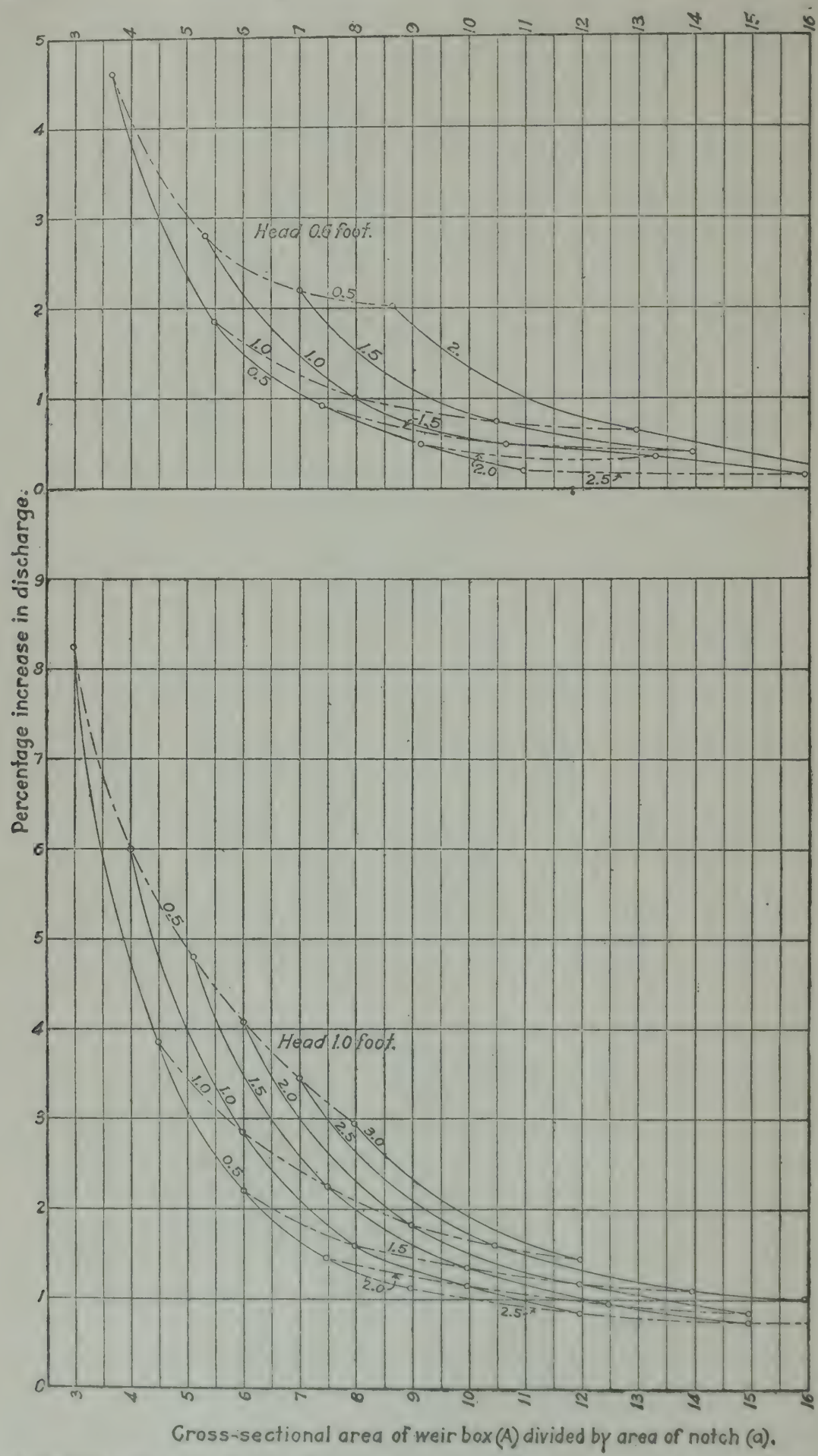

FIG. 16.—Curves showing the effect of different ratios of cross-sectional area of the weir box (*A*) to the area of the notch (*a*) upon discharges through a 1-foot rectangular notch with heads of 0.6 foot and 1 foot. Full lines show bottom contractions in feet; dot-dash lines show end contractions in feet.

This indicates that a mean velocity of one-third foot per second is allowable where an error of 1 per cent in discharge is permissible.

By superimposing upon the similar curves for Cipolletti notches the curves showing the effect of different end and bottom contractions upon the discharges through rectangular notches, it was found that the end-contraction distances for Cipolletti notches should be taken from about the middle point of the side of the notch instead of from the end of the crest, in order to make the results of the two types of notches comparable.

Since the minimum bottom and end contractions possible without increasing the discharges beyond an allowable limit increase with the increase of the head run, weir boxes should be designed so as to give discharges within the allowable limit when the highest head intended to be run over the notch is being run. Francis stated (5, p. 72 and 134):

> In order that the end contraction may be complete, the sill and sides of the weir must be so far removed from the bottom and lateral sides of the reservoir (weir box) that they may produce no more effect upon the discharge than if they were removed a distance infinitely great.

He concludes from his experiments than an end contraction of $1H$ and a bottom contraction of $2H$ are the least permissible in order that his formula may apply.

Smith (10, p. 120) gave the necessary end contractions as $3H$. He also suggested (p. 122) that the effect of contraction should not be confused with the effect of velocity of approach, which is so commonly done in taking the term "complete contraction" to include both the effect of contraction and the velocity of approach. Cipolletti (3, p. 23–24) accepted the results of the Francis experiments for end and bottom contractions. He also quotes a rule deduced by Lesbros from results of his (Lesbros's) experiments, that both contractions should be at least 2.7 times the depth of the nappe. Cipolletti (3), from the experiments of Francis (5), deduced the following: (1) When the end contractions equal $2H$ and the bottom contraction $3H$, the bottom and side walls no longer have any appreciable effect upon the discharges through the notch. This condition, he states, may cause an increase of about 0.15 per cent in the discharge. (2) With end contractions of $1.5H$ and a bottom contraction of $2.5H$ the increase in discharge would be about 0.5 per cent. (3) With end contractions of $1H$ and a bottom contraction of $2H$ the discharges will be increased about 1 per cent. He also takes account of the fact that the velocity of approach must not exceed a certain limit.

The ratio of the cross-sectional area of the weir box to the cross-sectional area of the notch necessary for complete contraction has been given by Carpenter (2, p. 29) as 7. The coefficient using this expression of ratio was proposed by J. Weisbach in 1845 and has been elaborated upon by a number of writers and experimenters (6, p. 312). Figure 16 indicates that there is no fixed value of the ratio A to a which will insure

complete contraction in all cases. It also indicates that the value of such ratio should be greater than 7 in all cases, and that 15 probably would come nearer than 7 to meeting average conditions.

EFFECT OF SUPPRESSING BOTTOM CONTRACTIONS WITH A 90° TRIANGULAR NOTCH

In order to throw more light upon the question of the effect of bottom contractions upon discharges through triangular notches (9, p. 114–116) experiments were made with a 90° triangular notch with the floor of the weir box at the same level as the vertex of the notch. The width of the weir box used was 10 feet, being the same as that in the standard test with complete contractions, but in the standard test the floor was about 4½ feet below the vertex of the notch. The discharges through the 90° triangular notch with the bottom contraction entirely suppressed was found to be represented by the formula $Q = 2.53H^{2.496}$, which varies but little from Thomson's formula for the flow through a 90° triangular notch having complete bottom contractions. It is probable that some part of the increased discharge obtained when the floor was level with the vertex of the notch was due to the increased velocity of approach. The increase in the discharges amounted to 1.6 per cent with a head of 1 foot, but gradually diminished as the head was decreased. The percentage of increase with heads of 0.3 foot or over is represented by the formula $E = 101.6H^{0.016} - 100$.

RELATION OF LENGTHS OF NOTCHES TO DISCHARGES

The principal advantage claimed in irrigation practice for Cipolletti notches over other notches has been that the discharges are proportional to the crest lengths. This claim is not in accordance with the limitation put on the notch by Francis and Cipolletti, but has been very generally made in irrigation practice. The failure of this theory is shown in Table XV, in which the discharges through Cipolletti and rectangular notches of different lengths are compared with the discharges through a 1-foot Cipolletti and a 1-foot rectangular notch, multiplied by the number of feet in length of the notches. The percentages in the table represent the failure of the larger notches to give discharges proportional to their lengths. It will be seen from the table that rectangular notches give discharges which are more nearly proportional to their lengths than do Cipolletti notches. The percentages of error increase with the head and length of the crest until the discharge through a 4-foot Cipolletti notch with a 1-foot head is 9.2 per cent less than four times the flow through a 1-foot notch with a 1-foot head, and the discharge through a 4-foot rectangular notch is 4 per cent greater than 4 times the discharge through a 1-foot rectangular notch with a 1-foot head. Side slopes of 1 to 4 are therefore too flat and vertical sides are too steep to give discharges proportional to the length of the crest.

TABLE XV.—*Relation of length to discharge (in cubic feet per second) of weirs*

RECTANGULAR NOTCHES

Head.	1-foot crest.	1.5-foot crest.				2-foot crest.				3-foot crest.				4-foot crest.			
	Discharge.	Discharge.	1.5 × discharge through 1-foot notch.	Difference.		Discharge.	2 × discharge through 1-foot notch.	Difference.		Discharge.	3 × discharge through 1-foot notch.	Difference.		Discharge.	4 × discharge through 1-foot notch.	Difference.	
				Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.
Feet.																	
0.20	0.291	0.439	0.437	0.002	0.5	0.588	0.582	0.006	1.0	0.887	0.873	0.014	1.6	1.187	1.164	0.023	2.0
.25	.404	.609	.606	.003	.5	.817	.808	.009	1.1	1.233	1.212	.021	1.7	1.650	1.616	.034	2.1
.30	.527	.796	.790	.006	.8	1.068	1.054	.014	1.3	1.612	1.581	.031	2.0	2.158	2.108	.050	2.4
.40	.804	1.214	1.206	.008	.7	1.630	1.606	.024	1.5	2.464	2.412	.052	2.2	3.299	3.216	.083	2.6
.50	1.113	1.684	1.670	.014	.8	2.262	2.226	.036	1.6	3.421	3.339	.082	2.5	4.583	4.452	.131	2.9
.60	1.453	2.201	2.180	.021	1.0	2.956	2.906	.050	1.7	4.474	4.359	.115	2.6	5.996	5.812	.184	3.2
.70	1.819	2.756	2.729	.027	1.0	3.705	3.638	.067	1.8	5.611	5.457	.154	2.8	7.522	7.276	.246	3.4
.80	2.210	3.351	3.315	.036	1.1	4.506	4.420	.086	1.9	6.828	6.630	.198	3.0	9.158	8.840	.318	3.6
.90	2.624	3.980	3.936	.044	1.1	5.354	5.248	.106	2.0	8.118	7.872	.246	3.1	10.891	10.496	.395	3.8
1.00	3.058	4.642	4.587	.055	1.2	6.247	6.116	.131	2.1	9.476	9.174	.302	3.3	12.716	12.232	.484	4.0

CIPOLLETTI NOTCHES

0.20	0.302	0.450	0.453	−0.003	−0.7	0.599	0.604	−0.005	−0.8	0.898	0.906	−0.008	−0.9	1.198	1.208	−0.010	−0.8
.25	.423	.628	.634	− .006	− .9	.836	.846	− .010	−1.2	1.252	1.269	− .017	−1.3	1.669	1.692	− .023	−1.4
.30	.557	.826	.835	− .009	−1.1	1.098	1.114	− .016	−1.4	1.642	1.671	− .029	−1.7	2.188	2.228	− .040	−1.8
.40	.866	1.277	1.299	− .022	−1.7	1.692	1.732	− .040	−2.3	2.526	2.598	− .072	−2.8	3.361	3.464	− .103	−3.0
.50	1.221	1.793	1.831	− .038	−2.1	2.370	2.442	− .072	−2.9	3.529	3.663	− .134	−3.7	4.691	4.884	− .193	−4.0
.60	1.623	2.371	2.434	− .063	−2.6	3.126	3.246	− .120	−3.7	4.644	4.869	− .225	−4.6	6.166	6.492	− .326	−5.0
.70	2.069	3.006	3.103	− .097	−3.1	3.955	4.138	− .183	−4.4	5.861	6.207	− .346	−5.6	7.772	8.276	− .504	−6.1
.80	2.559	3.700	3.838	− .138	−3.6	4.855	5.118	− .263	−5.1	7.177	7.677	− .500	−6.5	9.507	10.236	− .729	−7.1
.90	3.092	4.448	4.638	− .190	−4.1	5.822	6.184	− .362	−5.9	8.586	9.276	− .690	−7.4	11.359	12.368	−1.009	−8.2
1.00	3.667	5.251	5.500	− .249	−4.5	6.856	7.334	− .478	−6.5	10.085	11.001	− .916	−8.3	13.325	14.668	−1.343	−9.2

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For the purpose of throwing light upon what would be the probable shape of a type of notch the discharges through which, with the given head, would be proportional to the crest length, the data obtained for the 2-foot rectangular, the 2-foot Cipolletti, and the 2-foot trapezoidal

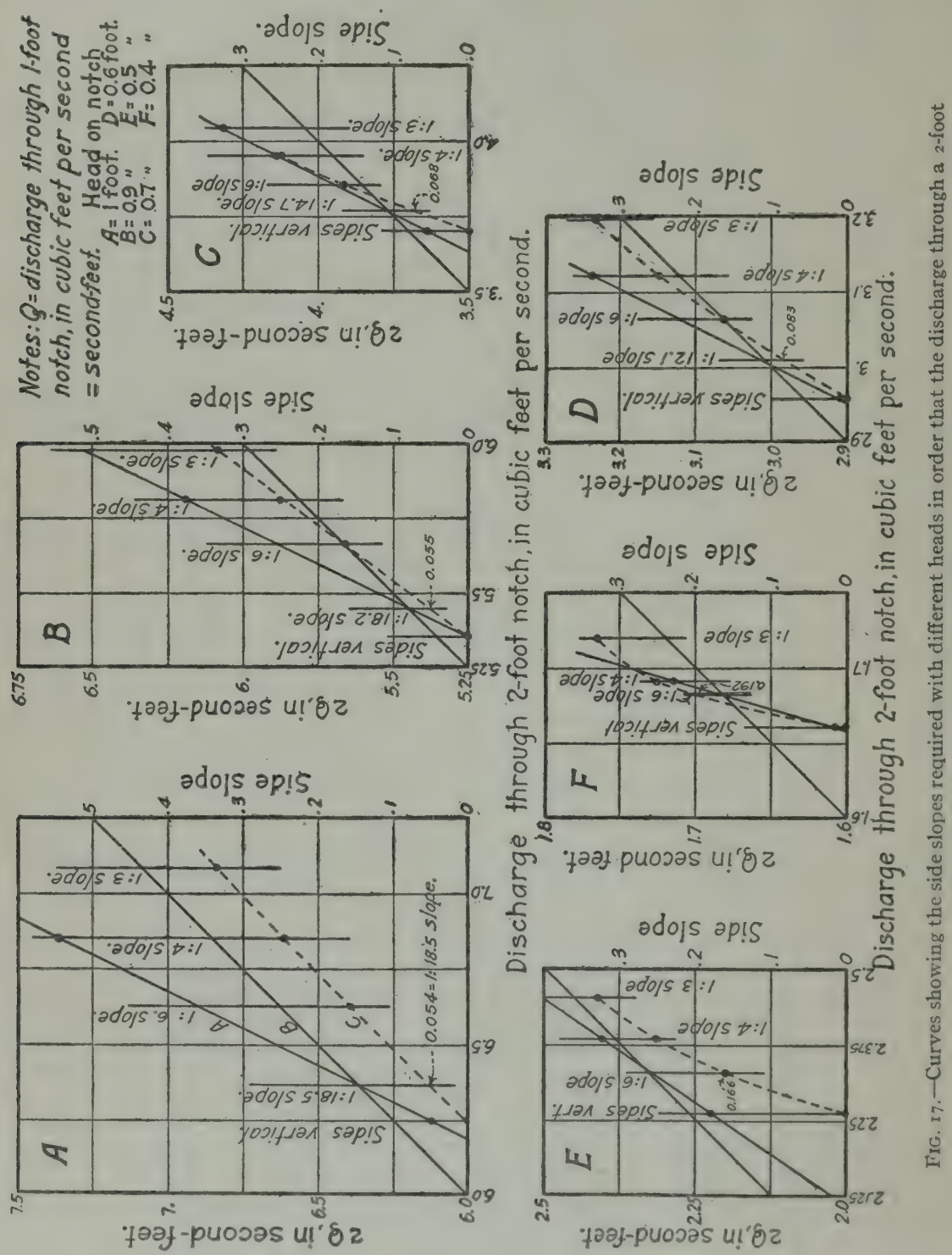

Fig. 17.—Curves showing the side slopes required with different heads in order that the discharge through a 2-foot notch will be twice the discharge through a 1-foot notch.

notches with side slopes 1 to 3 and 1 to 6 were plotted, a set of curves being made for each of the following heads: 0.4, 0.5, 0.6, 0.7, 0.9, and 1 foot (fig. 17). Lines *A* were obtained by plotting the actual discharges through the rectangular and Cipolletti notches with a given head against twice the discharges through 1-foot notches with the same head. Since no experiments were made with 1-foot notches having side slopes

1 to 3 and 1 to 6, it was assumed that similar plottings for such notches would lie on the same straight line as those for the rectangular and Cipolletti notches. Lines *B* pass through the origin and have a slope of 45°. The discharges through a 2-foot notch with the various heads that would fulfill the condition of being twice the discharge through a 1-foot notch with the same head must lie on this 45° line. Curves *C* were obtained by plotting the discharges through the 2-foot notches of different shapes against the decimal expression of the side slope of the notches.

In each set of curves the point of intersection with the *C* curve of a vertical line drawn through the point of intersection of lines *A* and *B* indicates the side slopes which are necessary with a given head in order that the discharge through a 2-foot notch shall be twice that through a 1-foot notch. The slopes found expressed as ratios of the horizontal to the vertical distance are given in Table XVI and indicate that the sides of a 2-foot notch which would give twice the discharges of a similar 1-foot notch with heads up to 1 foot at least must be curves and must approach the vertical as they go up.

TABLE XVI.—*Side slopes necessary in order that a 2-foot notch discharge twice the amount of water from a 1-foot notch*

Head.	Slopes.
Feet.	
1.0	1 to 18.5
.9	1 to 18.2
.7	1 to 14.7
.6	1 to 12.1
.5	1 to 6.5
.4	1 to 5.25
.2	[a] 1 to 4.0

[a] Obtained from data for 0.2 head.

No attempt was made to determine the exact shape of the sides of the notch. They would be so complex, however, that their construction would render impracticable the use of such notches on the farm. Because of the appreciable difference in the effects of contraction with notches of different sizes, a similar comparison of the discharges through larger notches with those through a 1-foot notch would probably give results different from those obtained for the 2-foot notch.

SUBMERGED RECTANGULAR AND CIPOLLETTI NOTCHES

A notch is said to be submerged or "drowned" when the water level on the downstream side is higher than the crest of the notch. To determine the effect of submergence upon the discharges 757 experiments were made with the 1-, 2-, 3-, and 4-foot rectangular and Cipolletti notches used in the free-flow experiments. The conditions on the up-

stream side of the weir were those of the standard weir box—that is, the width of box was 10 feet; the depth of the box 6 feet; and the distance of the floor from the crest of the notches about 4½ feet. A bulkhead was placed across the escape channel of the standard box, parallel to and about 5¾ feet from the plane of the weir, thus making the spill box 10 feet wide, 5¾ feet long, and 4 feet deep, the floor being about 2½ feet below the crest of the notch. The height of the water in the escape channel was controlled by a steel head gate 20 inches square with a vertical slide set in the middle of the bulkhead about 0.5 foot above the floor, and by a 4-inch gate valve set near one end of the bulkhead, the finer regulation being made with this valve. The elevation of the water in the escape channel was determined by a hook gauge set in the concrete gauge box, which was connected with the escape channel by two 1-inch pipes which entered near the floor line 3½ feet from the plane of the weir.

Several minutes were required to adjust the flow of the water before an experiment was started, but when the desired condition of flow had been obtained it was maintained without difficulty throughout the test, except when the head on the upstream side of the weir was high and the head on the downstream side was small. Under this condition the large volume of water flowing through the notch depressed the water surface immediately downstream from the notch. This was followed by a standing wave, and the resulting backlashing and surging in the escape channel caused intermittent pulsations in the hook-gauge still box. The errors, however, were largely compensating, as is indicated by the consistent curves obtained from the experimental data.

The discharges with different heads through the different notches, with free flow and with different depths of submergence, were plotted (figs. 18 to 25) with discharges in cubic feet per second as abscissas and the heads upstream from the weir (H_A) as ordinates. Curves were drawn showing the discharges with different heads upstream from the weir (H_A) with varying differences (H_D) between the head upstream from the weir (H_A) and the head downstream from the weir (H_B). The method of interpolating between the values given on the curves in figures 18 to 25 is indicated by the dotted lines in figure 18 and is based upon the fact that $H_A = H_B + H_D$. The $H_D = 0.15$ line must pass through the points where the various H_B lines intersect the H_A lines and satisfy the equation $H_A - H_B = 0.15$. The $H_B = 0.65$ line would be located similarly upon the points of intersection of the H_A and H_B lines. Interpolations for other depths of submergence can be made in the same manner by drawing H_A lines for other than even 0.05-foot heads. For the purpose of comparison, the free-flow discharge curve is drawn with each set of submergence curves.

A series of experiments was made to determine the effect upon discharges of changing the conditions in the escape channel from free flow

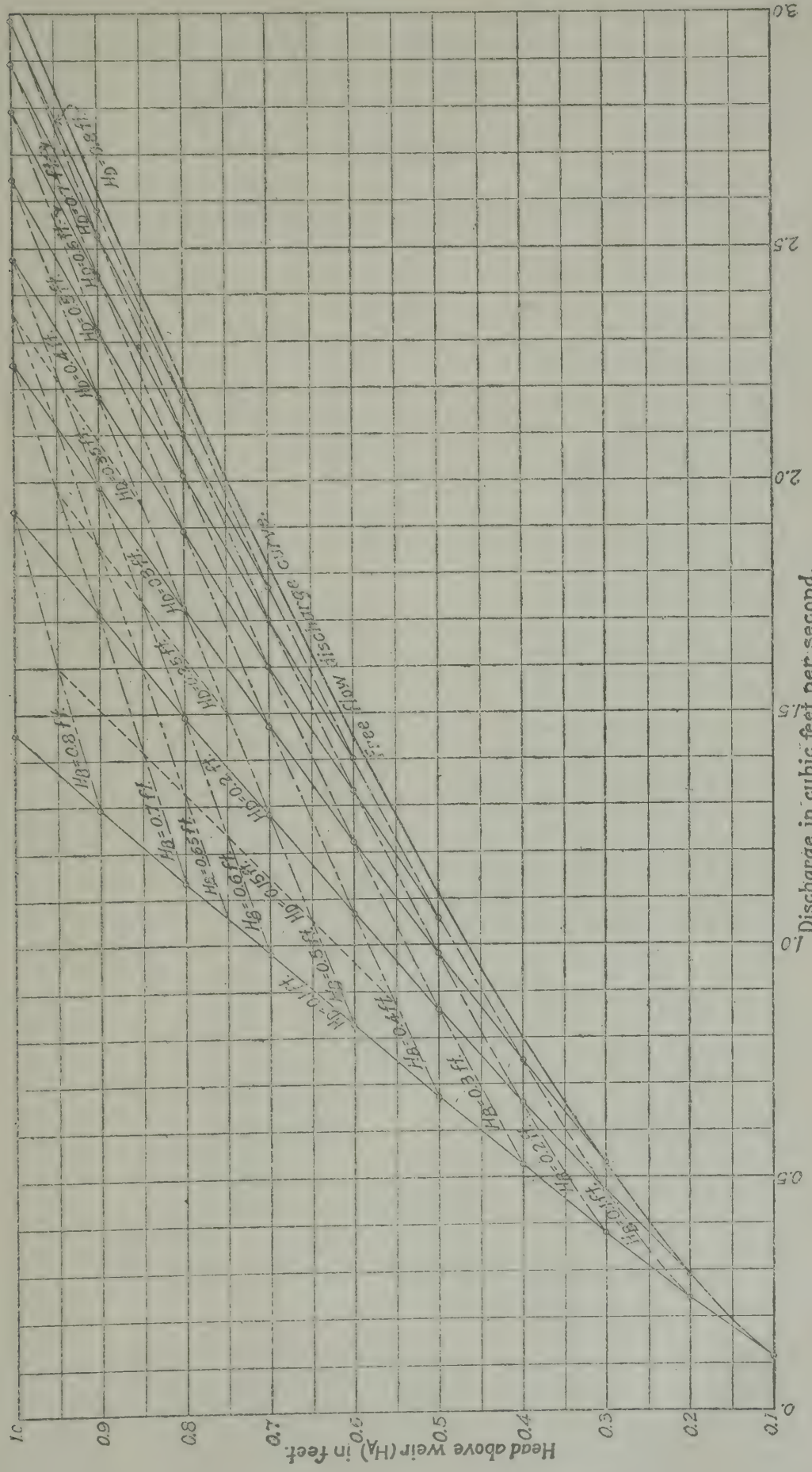

FIG. 18.—Curves showing the discharges through a 1-foot rectangular notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

to submergence. In this set of experiments the head upstream from the weir was made constant, but the conditions downstream were changed by stages in the runs from a free fall of 0.5 foot to a submergence of 0.1 foot. The discharges through this change of conditions remained the same within the limit of the experimental error—0.5 per cent. The

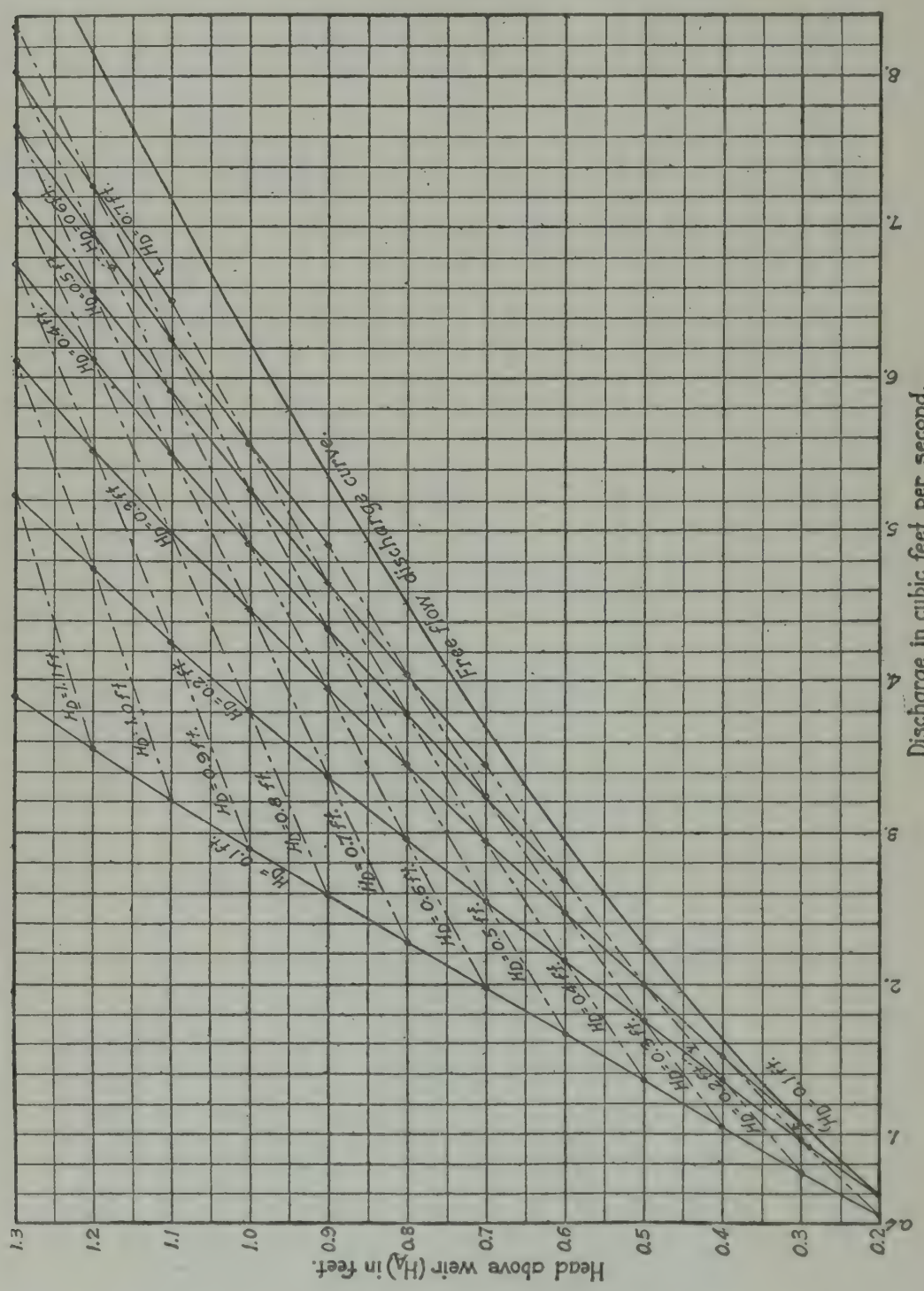

FIG. 19.—Curves showing the discharges through a 2-foot rectangular notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

notches were all thin-edged, the cross section of the weir box in every case was large enough for full-contraction conditions, and the escape channel was wide enough to allow the sheet of water to expand laterally after passing through the notch. In none of the tests was the amount of submergence small enough to make it possible to determine whether the discharge is actually increased with the small amounts of submer-

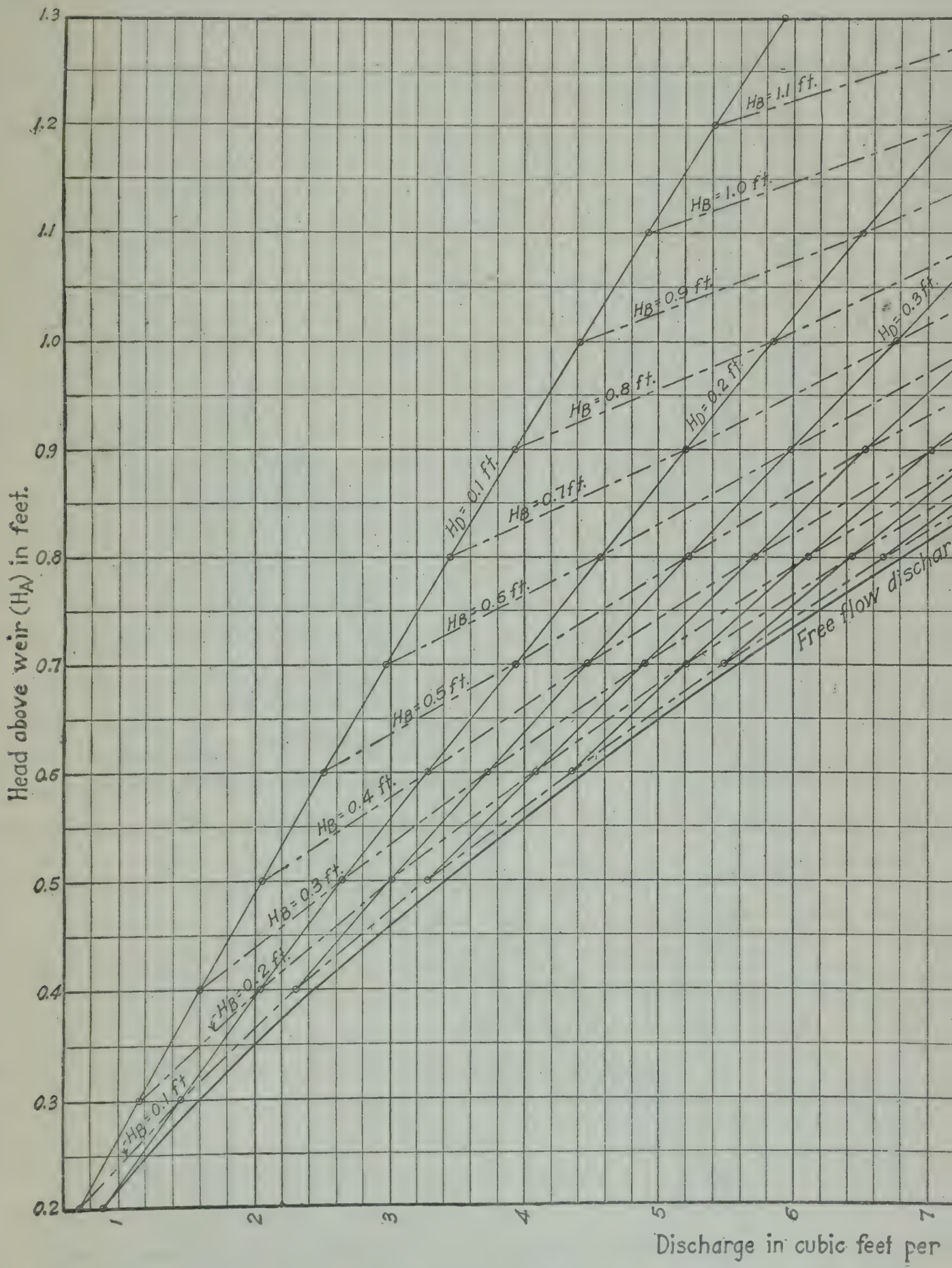

FIG. 20.—Curves showing the discharges through a 3-foot rectangular notch submerged to different dep

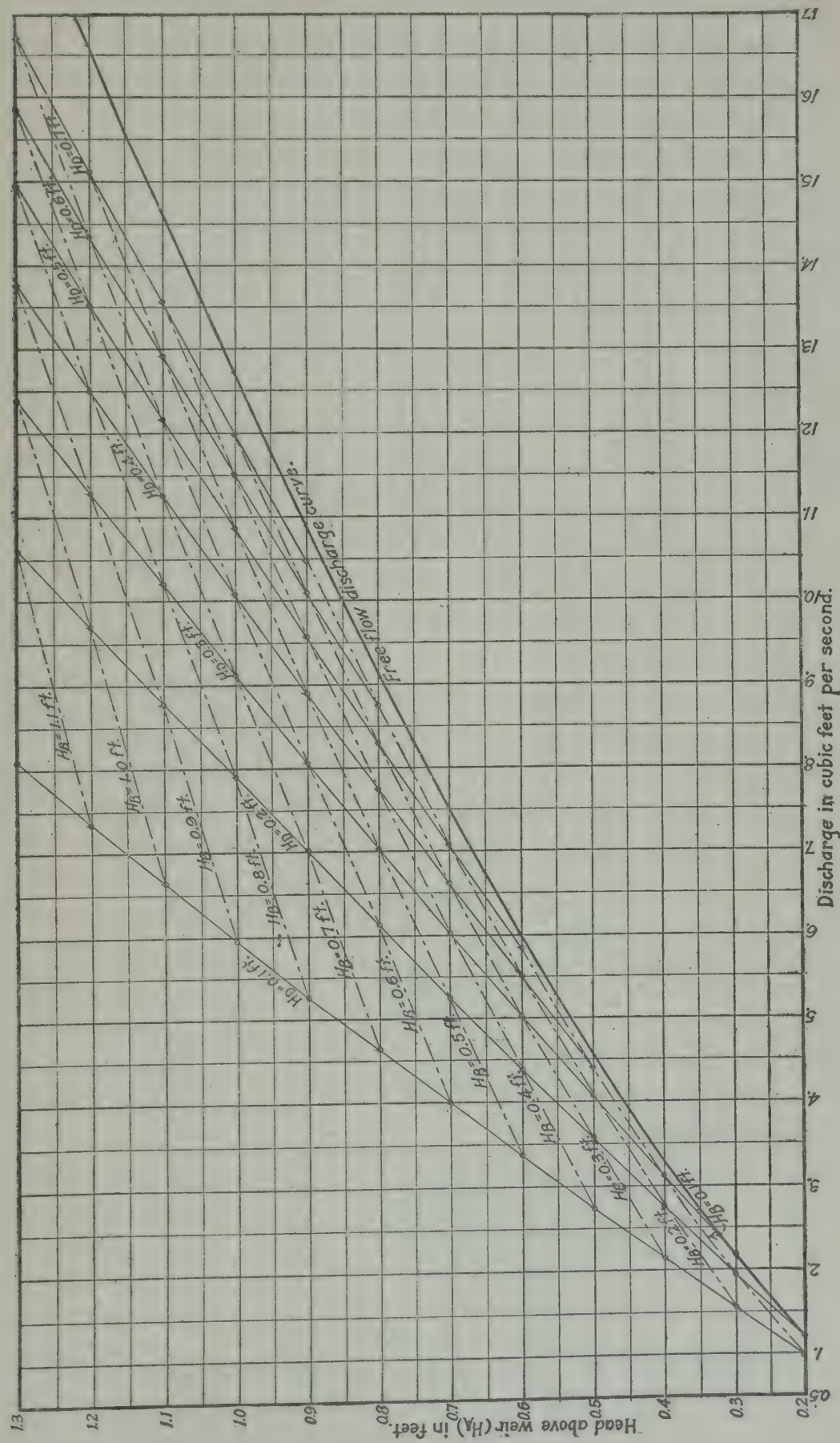

FIG. 21.—Graph showing the discharges through a 4-foot rectangular notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

gence. For all practical purposes, however, it may be stated that the discharge is not materially affected unless the notch is submerged until H_B is at least one-tenth of H_A. When H_B is one-eighth of H_A, the dis-

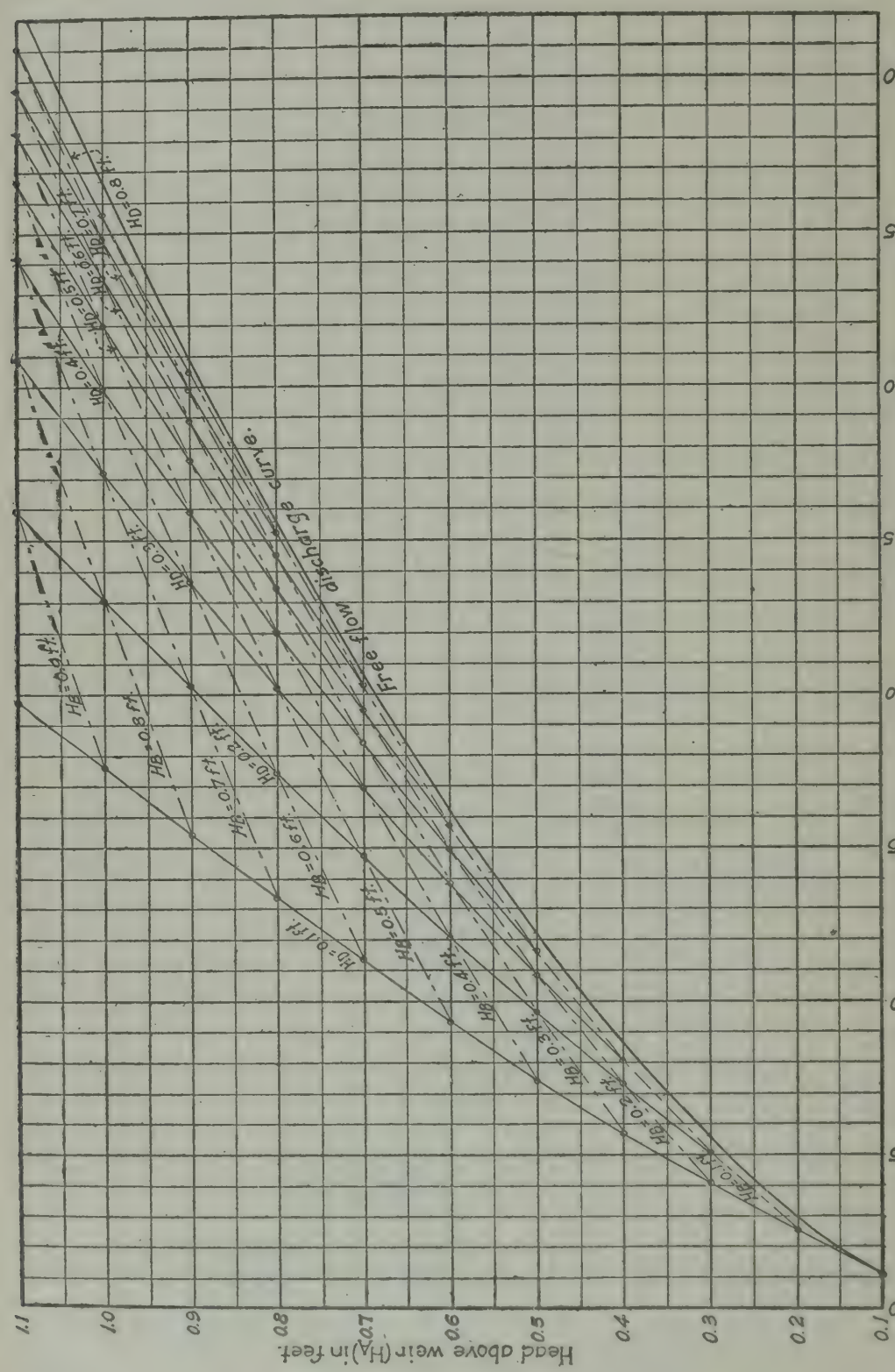

FIG. 22.—Curves showing the discharges through a 1-foot Cipolletti notch submerged to different depths. H_A = head above weir; H_B = head below weir; $H_D = H_A - H_B$ = effective head.

charge is decreased approximately 2 per cent; when it is one-fourth, the decrease is approximately 6 per cent; and when it is one-third, the decrease is approximately 9 per cent. These percentages vary somewhat with the head.

SUMMARY

(1) The discharges through rectangular and Cipolletti notches when plotted logarithmically do not give straight lines and therefore can not be represented correctly by a formula of the type $Q=CLH^n$. It was

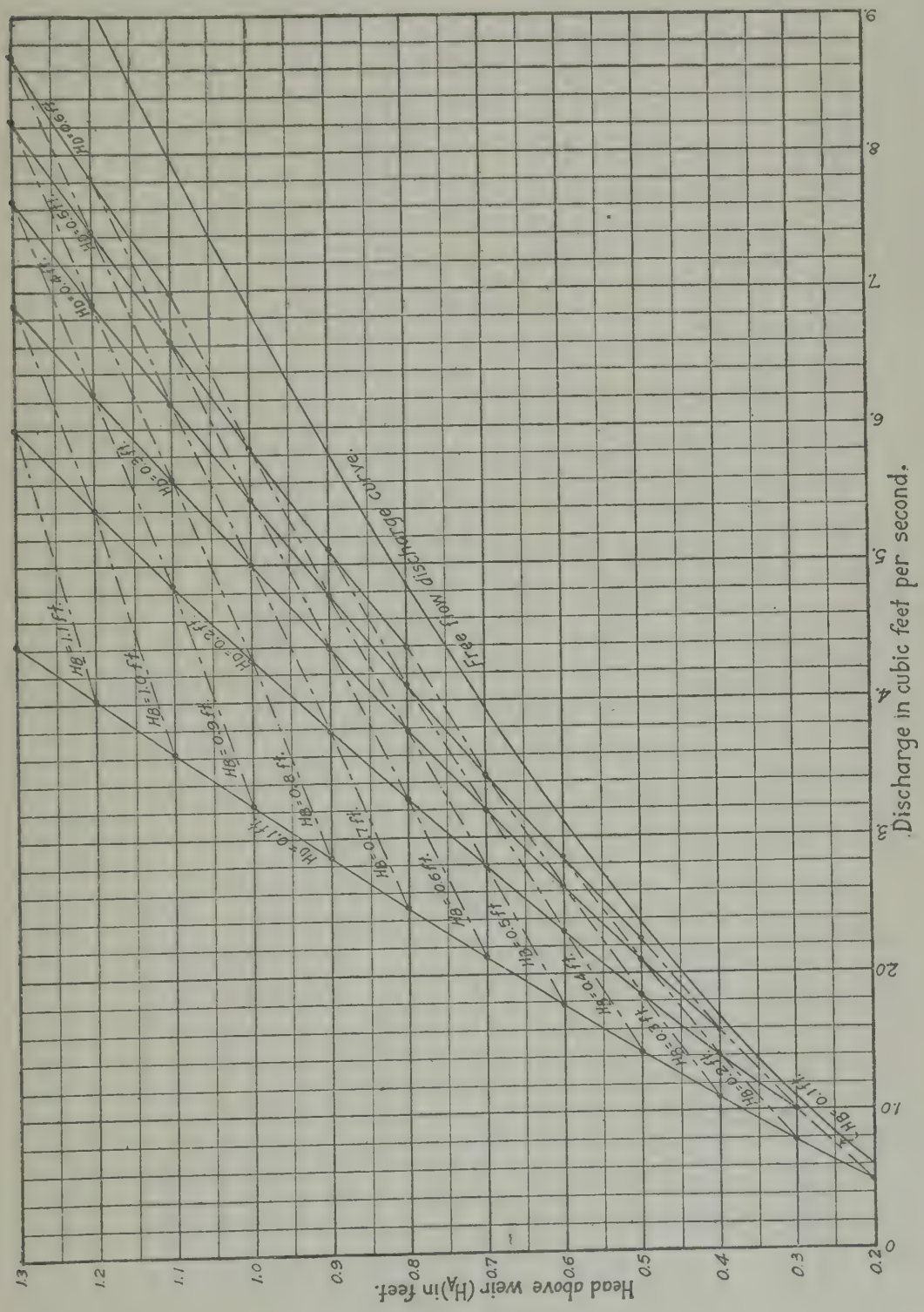

FIG. 23.—Curves showing the discharges through a 2-foot Cipolletti notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

found, however, in the case of the rectangular notches experimented with and the heads of water run, that a straight-line formula could be deduced that within the range of the experiments gave values quite close to the experimental data.

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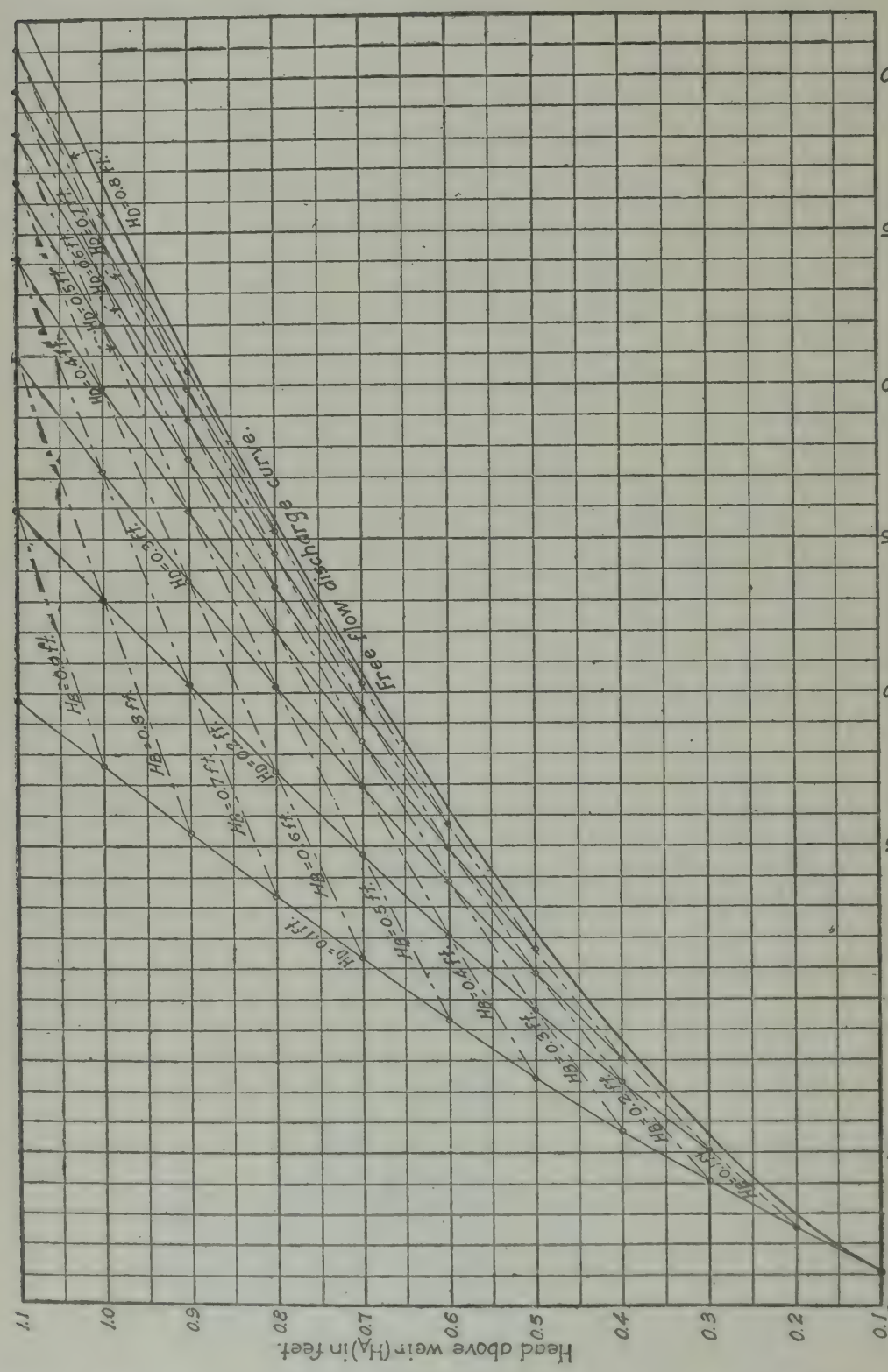

FIG. 22.—Curves showing the discharges through a 1-foot Cipolletti notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

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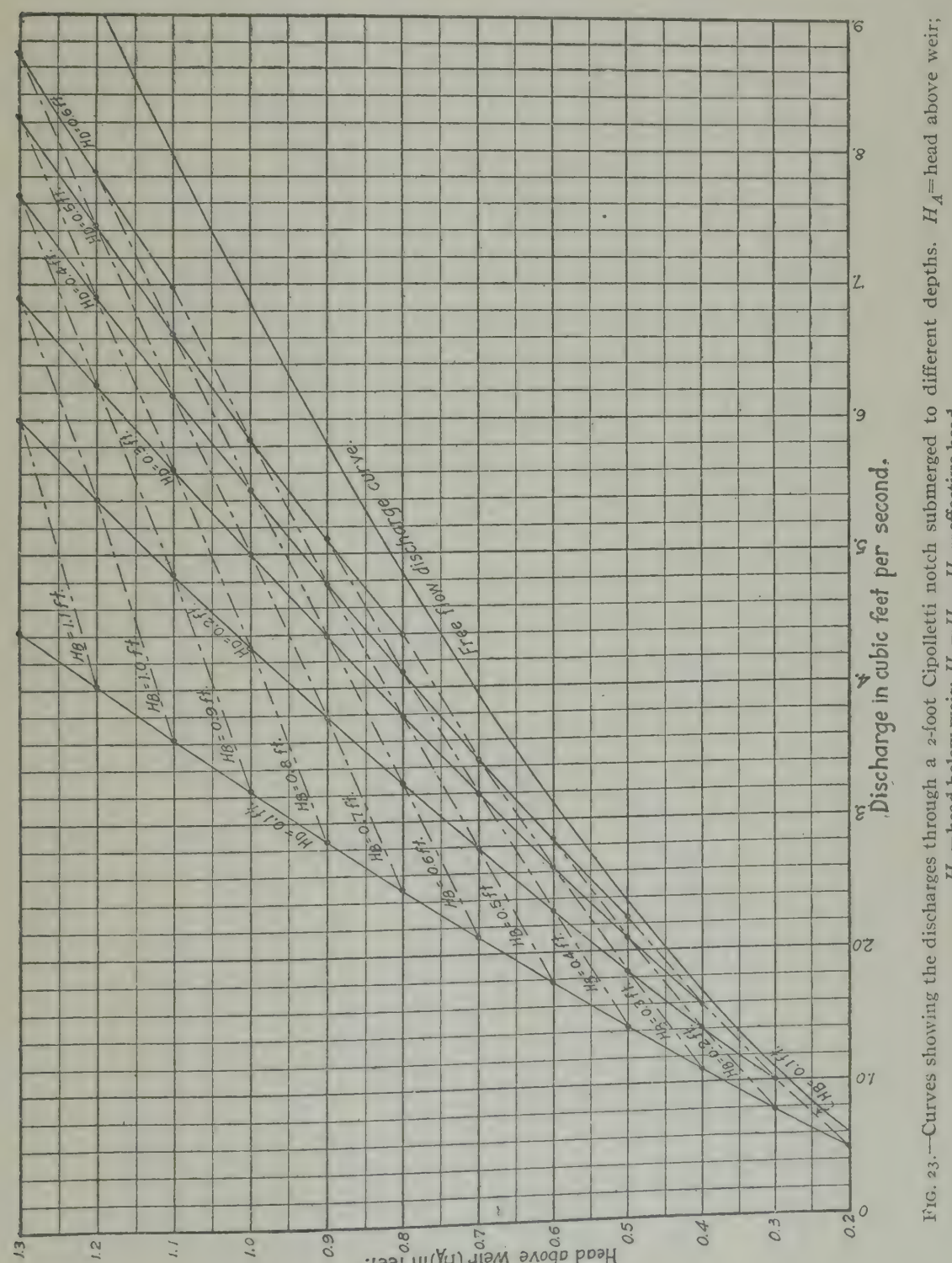

FIG. 23.—Curves showing the discharges through a 2-foot Cipolletti notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

found, however, in the case of the rectangular notches experimented with and the heads of water run, that a straight-line formula could be deduced that within the range of the experiments gave values quite close to the experimental data.

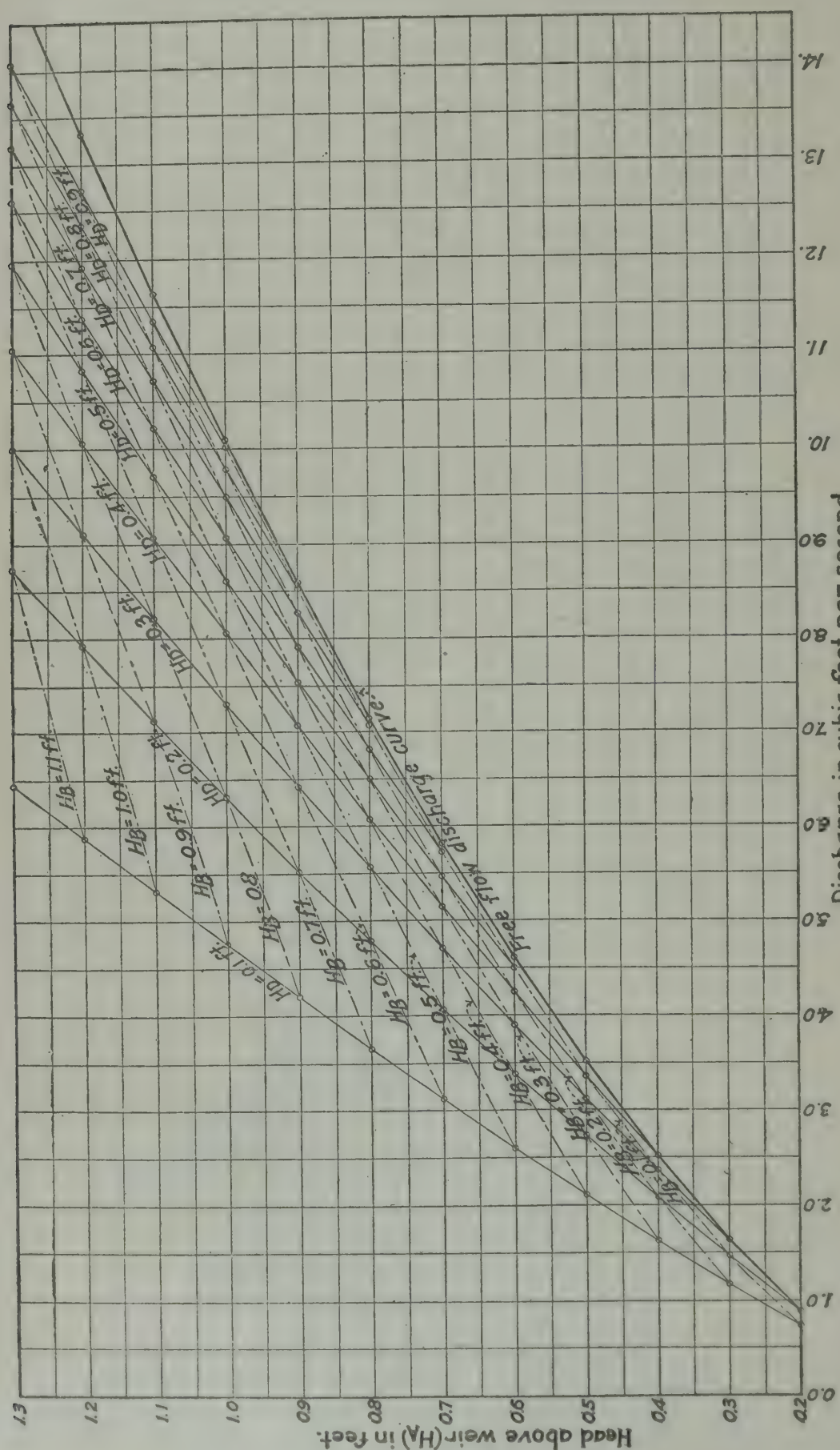

FIG. 24.—Curves showing the discharges through a 3-foot Cipolletti notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

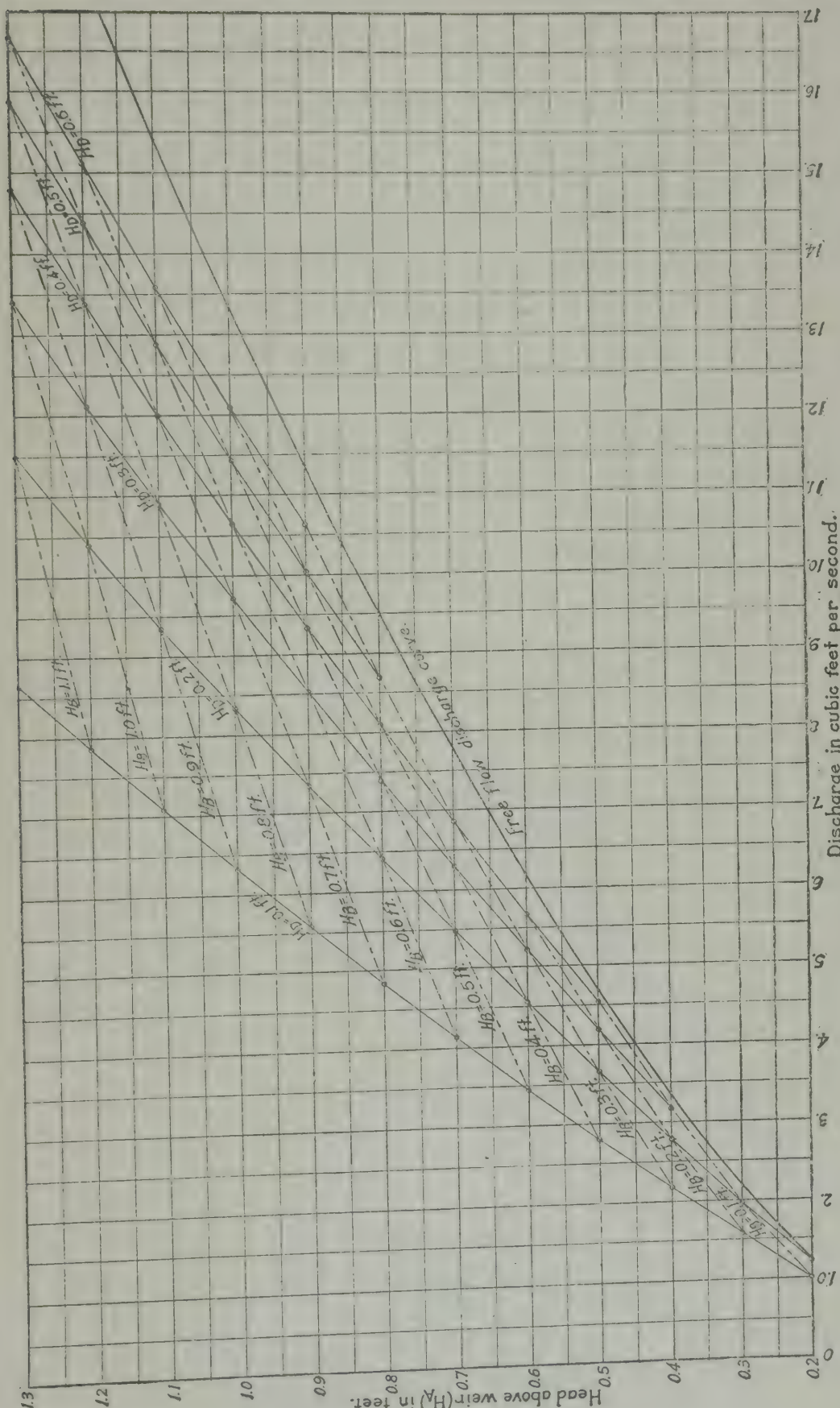

FIG. 25.—Curves showing the discharges through a 4-foot Cipolletti notch submerged to different depths. H_A=head above weir; H_B=head below weir; $H_D=H_A-H_B$=effective head.

(2) The formula

$$Q = 3.247LH^{1.48} - \left(\frac{0.566L^{1.8}}{1+2\ L^{1.8}}\right)H^{1.9}$$

gives discharge values for 1-, 1.5-, 2-, 3-, and 4-foot rectangular notches that agree within a maximum of approximately 1.2 per cent and within an average of 0.28 per cent with the curves plotted from the experimental data.

(3) The discharges through the 0.5-foot rectangular notch do not follow the same law as those for the longer notches. The formula

$$Q = 1.593H^{1.526}\left(1 + \frac{1}{800H^{2.3}}\right)$$

gives values consistent with the curve plotted from the experimental data.

(4) The Francis formula gives values within approximately 2 per cent of the actual discharges, so long as the head does not exceed one-third the length of the notch.

(5) Within the limits of the experiments the formula

$$Q = 3.08L^{1.022}\ H^{(1.46+0.003L)}$$

gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot rectangular notches that agree within a maximum of 0.7 per cent, and an average of 0.26 per cent, with the values given in the curves plotted from the experimental data.

(6) The formula $Q = 1.566H^{1.504}$ gives values for the 0.5-foot rectangular notch that agree within 1 per cent with the curves plotted from the experimental data.

(7) The curve-line formula for rectangular notches takes account of the law of variation of the discharge curves better than does the straight-line formula and, consequently, it appears that it will give closer values for higher heads and longer notches than those experimented with.

(8) The formula

$$Q = 3.247LH^{1.48} - \left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9} + 0.609H^{2.5}$$

gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot Cipolletti notches that agree within 0.5 per cent with the curves plotted from the experimental data, except in the case of the lower heads on the 1-foot notch, where the maximum divergence is approximately 1½ per cent.

(9) The discharges through the 0.5-foot Cipolletti notch do not follow the same law as those for longer notches. The formula

$$Q = 1.593H^{1.526}\left(1 + \frac{1}{800H^{2.3}}\right) + 0.587H^{2.53}$$

represents the discharges through such a notch.

(10) The Cipolletti formula gives discharge values within 1½ per cent of the actual discharges so long as the head does not exceed one-third the length of the crest of the notch.

(11) The formula

$$Q = 3.08L^{1.022}H^{(1.46+0.003L)} + 0.6H^{2.6},$$

which is based on the straight-line formula for rectangular notches, gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot Cipolletti notches that agree within a maximum of 1 per cent with the curves plotted from the experimental data, the divergences at all but a few points being 0.5 per cent or less. The formula for the 0.5-foot notch is $Q = 1.566H^{1.504} + 0.56H^{2.55}$.

(12) The Cipolletti type of notch does not give discharges as nearly proportional to the length of crest as does the rectangular type, consequently, since rectangular notches are simpler to construct and the formula for such notch gives as accurate discharge values as does the formula for Cipolletti notches, the rectangular-notch weir is to be preferred.

(13) The general formula for discharges through triangular notches of from 28° 4′ to 90°, and probably up to 109°, is

$$Q = (0.025 + 2.462\ S)H^{\left(2.5 - \frac{0.0195}{S^{0.75}}\right)}$$

where H is the head in feet and S the slope of the sides. Triangular notches having side slopes greater than about 1 to 4 (109°) are impractical, as the nappe adheres.

(14) The 90° triangular notch is the most practical triangular notch and should be used in preference to either rectangular or Cipolletti notches for discharges up to approximately 3 cubic feet per second. The approximate formula $Q = 2.49H^{2.48}$ will give discharge values for 90° notches which agree very closely with the value obtained with the general formula for triangular notches.

(15) The crest and sides of a weir notch need not be knife-edged. They are sufficiently sharp if the upstream corner of the edges is a distinct angle of 90° or less and the thickness of the edges is not so great that the water will adhere to them.

(16) The head should be measured upstream from the weir a distance of at least $4H$, or sidewise from the end of the crest in the plane of weir a distance of at least $2H$.

(17) The distances required for full contractions with rectangular and Cipolletti notches are approximately $2H$, but an additional cross-sectional area of the weir box is required to reduce the velocity of approach.

(18) With end contractions equal to $2H$ and a bottom contraction equal to $3H$, or end contractions equal to $3H$ and a bottom contraction equal to $2H$, the mean velocities of approach are about ⅓ foot

per second, and the discharges with medium to high heads do not agree more closely than approximately 1 per cent with the discharges computed by the formulas.

(19) The average ratio of the cross-sectional area of the weir box (A) to the cross-sectional area of the notch (a) required to give discharges within 1 per cent of the values obtained with the formula is greater than 7 and is probably near 15.

(20) In order to make the results comparable with those for rectangular notches, the end contractions for trapezoidal notches should be measured from about the middle point of the side of the notch, rather than from the end of the crest.

(21) A notch which would give discharges proportional to the lengths of the notches would probably have curved sides, the slope decreasing with the head.

(22) For all practical purposes, discharges through rectangular and Cipolletti notches are not affected until the notch is submerged to a depth equal to one-tenth the head upstream from the weir. Submergence equal to one-eighth the head upstream from the notch decreases the discharge approximately 2 per cent, that equal to one-fourth approximately 6 per cent, and that equal to one-third approximately 9 per cent.

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IDENTITY OF ERIOSOMA PYRI

By A. C. Baker,

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This paper has been written in order to reinstate the woolly aphis described by Fitch from apple (*Malus* spp.) roots, to point out its distinctness from the woolly apple aphis (*Eriosoma lanigerum* Hausmann), with which it has been confused, and to place it among the species of the genus to which it properly belongs.

In 1851 Fitch [1] described a woolly aphis under the name "*Eriosoma pyri.*" At the same time he described the work of what seems to be *E. lanigerum* Hausmann on apple. At the time of his original description Fitch evidently did not know of the genus Pemphigus. This is indicated from his remarks in his first report,[2] for in the description in this publication he is quite positive in placing his species in that genus. The description of the wingless forms agrees well, however, with *lanigerum.*

The identity of *pyri* has for many years been in doubt, and the name has been referred to different species as a synonym. The writer,[3] in his recent work on the woolly aphis, considered it to be *lanigerum.* This was based on two things: The description of the wingless forms, with the possibility of abnormality in the winged form, and Gillette's [4] statement in regard to the type. One fact, however, seems evident. The descriptions given by Fitch for his winged forms could not have been made from normal migrants of *lanigerum.* In fact, they could not have been made from winged forms of *lanigerum* at all. This is particularly true of the description in the first report.

Fitch's original notes on the species are now in the writer's hands, and they throw some interesting light on the question. After describing the wings minutely, Fitch says: "The wings serve best to distinguish this species, and an exact figure of one or both of them will be the best illustration of it that can be given," and again, "Neuration of the wings identical with that of *Myzoxylus imbricator.*" By 1871 Fitch had some feeling that his *pyri* might be a synonym of *lanigerum*, for in his notebook, under October 11 of that year, he suggests such a possibility. He adds, "My winged *lanigera* from Dr. Signoret is a Pemphigus, the 3rd vein being simple, but not so abortive at its base, and has all the veins slender."

[1] Fitch, Asa. Catalogue with references and descriptions of the insects collected and arranged for the State Cabinet of Natural History. *In* 4th Ann. Rpt. [N. Y.] State Cab. Nat. Hist., p. 68. 1851.

[2] —— [Report on the Noxious and Other Insects of the State of New York.] p. 7. *In* Trans. N. Y. State Agr. Soc., v. 14, 1854, p. 711. 1855. Reprint, p. 7, Albany, N. Y., 1856.

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Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. V, No. 23
Mar. 6, 1916
K—26

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This much remains: Fitch was not sure that he was not dealing with a compound species in his apple-root form and his winged forms. This is shown by the following note: "Amyot describes *Eriosoma lanigerum* as producing excrescences. Can these small lice be that species, and the winged ones another species accidentally present with them?"

What Fitch suspected is, the writer believes, true, and Fitch described the winged form of one species and the work of wingless *lanigerum*.

In the United States National Museum collection there is some material labeled "*P. pyri* Fitch, Type," and mounted by Pergande from the Fitch collection. This proves to agree in every detail with the different descriptions of the winged forms given by Fitch. There seems good reason to believe that the material represents the specimens from which Fitch drew up his diagnosis. This is strengthened by the fact that the species occurs in the vicinity of Washington, D. C., and Vienna, Va., upon apple and upon pear (*Pyrus* spp.) roots. It is particularly common upon pear roots, and it occurs also upon *Crataegus* spp. and ash (*Fraxinus* spp.).

Since this material seems to settle finally the standing of *pyri*, a description is here given of the form based upon this material and upon other specimens collected mostly from pear roots. The form proves to belong to the genus Prociphilus, and in order to separate it from other species of the genus, descriptive notes and figures are given of the other species known to the writer. Particular stress is laid in these notes on the dorsal wax plates of the thorax, since these seem to prove good diagnostic characters.

The writer has never seen specimens of *Prociphilus crataegi* Tullgren, and it may be possible that *pyri* and *crataegi* are the same, since the sensory characters are similar. There seems, however, to be considerable difference in measurements. The question as to their distinctness or identity can only be determined by a careful comparison of the two.

It is possible, also, that *venafuscus* Patch may prove to be *pyri*. But in the specimens studied by the writer the sensoria are much more even, and *pyri* seems to lack the small, pointed projection near the base of the third segment of the antennæ.

The following description will, however, serve to place *pyri*:

Prociphilus pyri (Fitch)

Fall migrant (fig. 1, *E*, ♀).—Morphological characters: Antennal segments as follows: I, 0.064 mm.; II, 0.096 mm.; III, 0.544 mm.; IV, 0.224 mm.; V, 0.24 mm.; VI, base 0.192 mm., unguis 0.064 mm.; segments III to VI with transverse sensoria, usually very irregular in disposition and giving the segments, particularly segment III, a gnarled appearance; segment III with 28 to 35 sensoria, segment IV with 8 or 9, segment V with about the same number, and segment VI with 3 to 6. These sensoria are on the underside of the antennæ, the upper surface being armed with a few hairs situated on tubercles. Head above with two oval or almost circular transparent wax plates. Dorsum of thorax with a pair of rather small, somewhat triangular wax plates. Forewings 4.38 mm. long and 1.43 mm. wide at their greatest width. Hind tibiæ 1.2 mm. long. Length from vertex to tip of cauda, 2.48 mm.

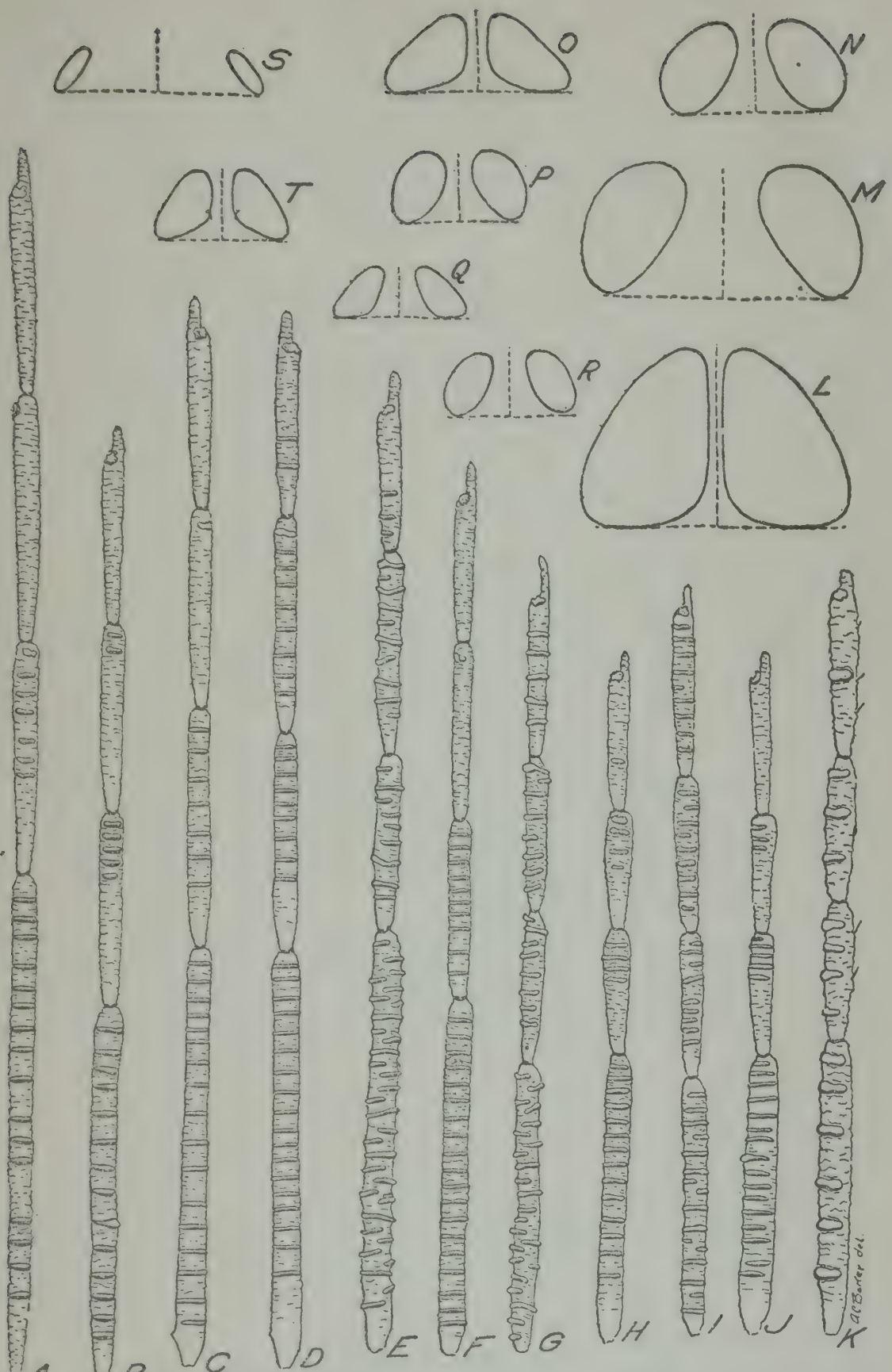

FIG. 1.—Structural characters of the species of Prociphilus. *A*, *P. bumulae:* Distal segments of antenna of spring migrant. *B*, *P. poschingeri:* Distal segments of antenna of spring migrant. *C*, *P. venafuscus:* Distal segments of antenna of spring migrant. *D*, *P. venafuscus:* Distal segments of antenna of fall migrant. *E*, *P. pyri:* Distal segments of antenna of fall migrant. *F*, *P. xylostei:* Distal segments of antenna of spring migrant. *G*, *P. populiconduplifolius:* Distal segments of antenna. *H*, *P. corrugatans:* Distal segments of antenna of spring migrant. *I*, *P. corrugatans:* Distal segments of antenna of spring migrant. *J*, *P. alnifoliae:* Distal segments of antenna. *K*, *P. tessellatus:* Distal segments of antenna. *L*, *P. bumulae:* Thoracic wax plates. *M*, *P. poschingeri:* Thoracic wax plates. *N*, *P. xylostei:* Thoracic wax plates. *O*, *P. venafuscus:* Thoracic wax plates. *P*, *P. corrugatans:* Thoracic wax plates. *Q*, *P. pyri:* Thoracic wax plates. *R*, *P. alnifoliae:* Thoracic wax plates. *S*, *P. populiconduplifolius:* Thoracic wax plates. *T*, *P. tessellatus:* Thoracic wax plates.

Color characters: Eyes, antennæ, and legs black; head black; prothorax and abdomen dull olive green with darker green marginal patches on the abdomen. Thoracic lobes and sternal plate black. Wing veins dark, with dusky bordering; the entire wing often more or less smoky. Head and thorax with a bluish white bloom; abdomen with a long cottony secretion, most pronounced caudad.

Prociphilus aceris (Monell).

Specimens of this species have a pair of large circular wax plates upon the head, and the dorsal wax plates of the thorax are of the same size and shape as those of *venafuscus* Patch. The sensoria on the third segment of the antennæ are oval in shape, some almost circular. They are thus not typical for the genus, but approach those of *attenuatus* Osborn and Sirrine for which Dr. E. M. Patch, of the Maine Experiment Station, has erected the genus Neoprociphilus. There seems to be, however, a gradual gradation from the type to this species. The wing also suggests that of *attenuatus*, and there is some doubt in the writer's mind in regard to the distinctness of Neoprociphilus. The measurements of antennal segments are as follows: III, 0.416 mm.; IV, 0.256 mm.; V, 0.24 mm.; VI, base 0.272 mm., unguis 0.048 mm.

Prociphilus alnifoliae (Williams) (fig. 1, *J*, *R*).

Alnifoliae is a species of medium size with rather short antennæ. The sensoria do not, as a rule, extend entirely across the segments, and they are often acute at each end, thus touching the margins of the segments as a point. The dorsal wax plates of the thorax are quite similar to those of *corrugatans*, being small and oval.

Prociphilus bumulae (Schrank) (fig. 1, *A*, *L*).

This species is very large and the sensoria of the antennæ are even and do not usually extend beyond the margins of the segment. The dorsal wax plates of the thorax are large and triangular and situated close together. In some specimens they almost touch along the median line. The measurements of antennal segments are as follows: III, 0.704 mm.; IV, 0.32 mm.; V, 0.32 mm.; VI, base 0.288 mm., unguis 0.064 mm.

Prociphilus corrugatans (Sirrine) (fig. 1, *H*, *I*, *P*).

This insect is a rather small species with regular sensoria present on the antennæ of the spring migrant, but with them irregularly arranged on the antennæ of the fall migrant. The dorsal wax plates of the thorax are small and oval in outline. The measurements of the antennal segments are: III, 0.32 mm.; IV, 0.144 mm.; V, 0.16 mm.; VI, base 0.128 mm., unguis 0.032 mm.

Prociphilus fraxini-depetalae (Essig).

This species appears to be a synonym of *venafuscus* Patch.

Prociphilus imbricator (Fitch).

This well-known species has not been figured. The sensoria of the antennæ are rather large, approaching those of *tessellatus* (Fitch). The dorsal wax plates of the thorax are small and well separated. The measurements of antennal segments are as follows: III, 0.368 mm.; IV, 0.176 mm.; V, 0.176 mm.; VI, base 0.192 mm., unguis 0.048 mm.

Prociphilus populiconduplifolius (Cowen) (fig. 1, *G*, *S*).

The antennæ of this species are characteristic in that the sensoria extend past the edges of the segments and give them an irregular or beaded effect on the margins. The wax plates on the thorax are also very characteristic, being minute and very widely separated. The antennal measurements are as follows: III, 0.4 mm.; IV, 0.288 mm.; V, 0.208 mm.; VI, base 0.208 mm., unguis 0.064 mm.

In the writer's opinion there is not sufficient difference for the retention of the genus Thecabius. The habits of the stem mothers may be different, as indicated by *patchii* Gillette, and yet the insects are very close in structure. The wax plates and sensoria vary greatly within the genus.

Prociphilus poschingeri (Holzner) (fig. 1, *B*, *M*).

Placed usually as a synonym of *bumulae* Schrank, this form as represented by our specimens shows some differences. The insects are considerably smaller and the dorsal wax plates of the thorax are not triangular and close together as are those of *bumulae*, but are considerably separated and oval in outline. Measurements of antennal segments: III, 0.406 mm.; IV, 0.246 mm.; V, 0.246 mm.; VI, base 0.224 mm., unguis 0.048 mm.

Prociphilus tessellatus (Fitch) (fig. 1, *K*, *T*).

The antennæ of *tessellatus* are hardly typical for this genus. The species seems, however, to fit here as well as anywhere. The sensoria on the antennæ are very broad for the genus and the shape of the segments is not typical. The dorsal wax pores are, however, quite normal. They are somewhat triangular in shape and are somewhat smaller than those of *venafuscus*. In many specimens each is armed with a small hair. Measurements of antennal segments: III, 0.4 mm.; IV, 0.171 mm.; V, 0.171 mm.; VI, base 0.197 mm., unguis 0.032 mm.

Prociphilus venafuscus (Patch) (fig. 1, *C*, *D*, *O*).

The form described by Dr. Patch[1] is the most typical American species and the antennal characters are very similar to those of *bumulae* Schrank. The clouding of the wings met with in *venafuscus* is present also in our specimens of *poschingeri* though it is not noted in those of *bumulae*. The dorsal wax plates of the thorax are, in *venafuscus*, triangular like those of *bumulae*. They are, however, very much smaller. Measurements of antennal segments: III, 0.56 mm.; IV, 0.288 mm.; V, 0.288 mm.; VI, base 0.224 mm., unguis 0.049 mm.

Prociphilus xylostei (De Geer) (fig. 1, *F*, *N*).

Specimens of this species are much smaller than those of *bumulae* or even those of *venafuscus*. The antennal characters are very similar to those of *venafuscus*. The dorsal wax plates of the thorax are, however, of quite different shape in the two species, although they are almost equal in size. Measurements of antennal segments: III, 0.48 mm.; IV, 0.24 mm.; V, 0.24 mm.; VI, base 0.197 mm., unguis 0.048 mm.

The average number of sensoria on the antennæ of the species figured is shown in the illustration. The number varies somewhat in different individuals.

[1] Patch, Edith M. Aphid pests of Maine. *In* Maine Agr. Exp. Sta. Bul. 202, p. 174. 1912.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., MARCH 13, 1916 NO. 24

A NEW PENETRATION NEEDLE FOR USE IN TESTING BITUMINOUS MATERIALS

By CHARLES S. REEVE, *Chemist*, and FRED P. PRITCHARD, *Assistant Chemist, Office of Public Roads and Rural Engineering*

During the early period of the bituminous paving industry the asphaltic cement was usually tested by chewing a small piece and judging its consistency by its resistance to the teeth. With the development of the industry and specifications for work of this character it soon became evident that some more definite method of determining and defining consistency must be evolved, and in 1889 H. C. Bowen, of Columbia University, first described [1] a machine for the purpose. This was followed some years later by the machines designed by A. W. Dow [2] and by Richardson and Forrest.[3]

All of these machines had for their basic principle the depth to which a No. 2 sewing needle would penetrate the material under certain specified conditions of load, time, and temperature. Most, if not all, needle manufacturers produce No. 2 sewing needles, all makes of which are not necessarily of the same shape and size. Since it has, however, been generally understood that the No. 2 needle manufactured by R. J. Roberts was that most often used for the selection of standard needles, the subcommittee of the American Society for Testing Materials which has the penetration test under investigation made the following recommendation in 1915: [4]

> The needles for this test shall be R. J. Robert's Parabola Sharps No. 2. They shall be carefully selected by the use of a hand glass, rejecting all that are manifestly of unusual shape or taper. Needles thus selected shall be compared with a standard

[1] Bowen, H. C. An apparatus for determining the relative degree of cohesion of a semi-liquid body. *In* School Mines Quart., v. 10, no. 4, p. 297–302, 2 fig. 1889.

[2] Dow, A. W. The testing of bitumens for paving purposes. *In* Amer. Soc. Testing Materials, Proc. 6th Ann. Meeting, 1903, v. 3, p. 354. 1903.

[3] Richardson, Clifford, and Forrest, C. N. The development of the penetrometer as used in the determination of the consistency of semi-solid bitumens. *In* Amer. Soc. Testing Materials, Proc. 10th Ann. Meeting, 1907, v. 7, p. 626–631, 3 fig. 1907. Discussion, p. 632–637.

[4] Report of sub-committee on penetration. *In* Amer. Soc. Testing Materials, Proc. 18th Ann. Meeting, 1915, v. 15, pt. 1, p. 353. 1915.

needle and further rejections made of those which vary more than one point from that obtained with the standard needle, on a sample having a penetration of approximately 60.

The committee further stated that it did not think it advisable to recommend at the present time a standard needle for reference, deferring such action until the next annual meeting of the society. Until such recommendation is made needles furnished with penetration machines are to be considered standard.

The above-recommended practice is representative of the method for selecting needles which has been followed in the Office of Public Roads and Rural Engineering, and the standard used for comparison and selection was a needle originally supplied with the penetration machine in use. It has been, however, not uncommon practice in certain laboratories to purchase a package of No. 2 needles and to use them on the assumption that they possessed the requisite dimensions and shape. In an effort to prove the fallacy of such an assumption the authors have taken an enlarged photograph of a package of Roberts's No. 2 needles, an examination of which will serve to make clear the ordinary variations in point, shape, and taper (Pl. LXXXII, fig. 1).

These variations are more clearly shown through a consideration of the results obtained in selecting needles to be used for routine testing in the office. Several packages were first sorted with the aid of a magnifying glass and micrometer caliper, and a selection made of those whose shape and size appeared to be identical with the shape and size of the standard. From a lot of 72 needles, only 12 were thus selected. From these 12, those were selected for use which gave practically identical results in the penetrometer with a so-called standard needle on two samples of oil asphalt. The results of these tests are given in Table I, from which it may be seen that only 5 of the 12 needles fulfilled the requirements. Needles that failed to give accepted values on the harder materials were not tried on the softer.

Inasmuch as only 5 needles out of 72 proved acceptable, it may be seen what results would follow from the indiscriminate use of No. 2 needles as such.

It is further to be noted particularly that there is no existing single standard with which comparison can be made, owing to the fact that there is no means of accurately defining or gauging the type of needle in use. The work herein described was undertaken for the purpose of devising, if possible, a needle which would give results practically identical with results now obtained in using the so-called standard needles, and which could be accurately described and duplicated at any time.

The standard needle on file in the office is 1.8 inches in length, with a diameter of 0.040 inch for a length of 1 inch from the eye. The remainder of the needle tapers in a parabolic curve to a sharp point. The simpler

needle to define would be one having a straight taper. Round, polished, annealed-steel drill rods having diameters of 0.042 inch were therefore cut into 2-inch lengths and pointed at one end with tapers having a length of $\frac{3}{16}$, $\frac{1}{4}$, $\frac{5}{16}$, and $\frac{3}{8}$ inch. Each needle was tempered and highly polished, then tested in the penetrometer on a material showing a penetration of 140 with the standard needle. The penetrations were as follows on needles made from 0.042-inch drill rod: $\frac{3}{16}$-inch taper, 125; $\frac{1}{4}$-inch taper, 127; $\frac{5}{16}$-inch taper, 129; $\frac{3}{8}$-inch taper, 134.

TABLE I.—*Results of a standardization test of penetration needles on oil asphalt*

[Accepted values 6.8, 6.9, 7.0]

Needle No.	Oil asphalt 1.					Oil asphalt 2.	
	Operator C.	Operator F.	Operator D.	Operator A.	Operator E.	Operator C.	Operator F.
Standard	6.9	6.9	6.9	7.0	7.0	15.9 15.6	15.7 15.6
1 (rejected)	7.2	7.3	.....	.....	.....	.....	.....
2 (O. K.)	6.8	6.6	.....	.....	6.8 6.9	15.9	15.9 .....
3 (O. K.)	6.9	6.7	.....	6.85	.....	15.8	15.8
4 (rejected)	7.1	7.4	.....	.....	.....	.....	.....
5 (rejected)	7.1	7.0	.....	.....	7.1	.....	.....
6 (O. K.)	6.7 6.7	6.95 6.95	6.8	6.95	.....	15.9	15.9
7 (rejected)	6.8	6.6	.....	6.55 6.3	.....	.....	.....
8 (rejected)	6.9	6.5	.....	.....	6.7	.....	.....
9 (rejected)	6.8 6.7	6.6	.....	6.55	.....	.....	.....
10 (O. K.)	6.9	6.75 6.75	.....	.....	6.8	16.0	16.0
11 (O. K.)	6.9	6.85	.....	.....	.....	15.9 16.0	16.0 .....
12 (rejected)	6.8	6.6	.....	.....	6.4 6.75	.....	.....

While none of these needles yielded as high results as the standard, the one showing the highest values was tested on a sample of material having a penetration of 95 with the standard needle. A penetration of 103 was obtained. This eliminated the 0.042-inch drill rod from further consideration, since it was evident that a taper which would check with the standard needle on softer materials would give higher results than the standard on harder materials.

Drill rod with a diameter of 0.041 inch was then tried. This actually measured 0.0405 inch, and the finished and polished needle from it had a diameter of 0.040 inch. Three pieces of that diameter were given tapers of $\frac{3}{16}$, $\frac{1}{4}$, and $\frac{5}{16}$ inch, respectively, then polished and tested in comparison with the standard needle. The results on four samples of bituminous materials are given in Table II.

TABLE II.—*Results of an asphalt penetration test with a needle made from a steel drill rod 0.041 inch in diameter*

Taper of needles.	Sample No. 5284 (blown oil asphalt).	Sample No. 5963 (oil asphalt).	Sample No. 5985 (blown oil asphalt).	Sample No. 8928 (fluxed native asphalt).
Standard	30	153	75	109
3/16-inch taper	30	148	70	106
1/4-inch taper	32	150	74	109
5/16-inch taper	34	153	80	112

It will be noted from the above that on all four samples, representing three different types of material, the needle with 1/4-inch taper gave results in comparatively closer accord with those obtained by the standard needle than did the others. Three new needles of this type were therefore made and tested in comparison with the standard needle on various types of bituminous material having a wide range of penetration. The results are given in Table III. When it was found that all three needles checked with the standard throughout, the No. 1 new needle was run comparatively with the standard on six additional products, covering a still wider range of materials, in order to determine whether products varying in their general adhesive character might have any effect on the results. It will be noted by referring to Table III that the needle which the writers have designed yields in all cases results practically identical with those obtained with the standard needle. In cases where no results are given for the No. 3 needle the omission is due to the fact that the samples were run before the third needle had been prepared. In all cases but one the results are given by two operators.

TABLE III.—*Results of a comparative test of the new penetration needle with a standard needle*

Sample No.	Material.	Standard needle.		Needle No. 1.		Needle No. 2.		Needle No. 3.	
		Operator A.	Operator B.	Operator A.	Operator B.	Operator A.	Operator B.	Operator A.	Operator B.
5959	Blown Texas oil asphalt	8	8	9	9	8	8	.....	.....
5284	.....do	30	31	32	32	32	32	.....	.....
8233	Mexican oil asphalt	41.5	41.5	43	41.5	41	41	.....	.....
8961	California oil asphalt	49	47	47	48	48	49	49	47
6811	Texas oil asphalt	77	76	77	78	76	76	.....	.....
8916	.....do	93	92	95	95	93	93	.....	.....
8962	California oil asphalt	94	94	94	94	93	93	94	94
6291	Texas oil asphalt	108	110	110	110	109	108	.....	.....
5406	Oil asphalt (cut-back)	114	114	111	111	114	114	113	112
8966	Mexican oil asphalt	118	121	118	120	117	117	117	119
8970	.....do	119	118	117	119	119	119	118	119
8963	California oil asphalt	135	133	134	133	135	133	135	136
5381	Oil asphalt (cut-back)	133	134	135	135	135	135	134	135
5963	Texas oil asphalt	151.5	150	150	151	151.5	151	.....	.....
5559	Oil asphalt (cut-back)	168	170	170	170	170	168	170	169
8963A	Fluxed California asphalt	192	193	192	194	195	196	195	193
8963B	.....do	236	239	235	236	234	238	233	234
8963C	.....do	292	295	295	.....	295	.....	291	.....
5118	Fluxed Trinidad asphalt	83	85	83	85	.....	.....	.....	.....
5119	Fluxed Cuban asphalt	65	65	65	67	.....	.....	.....	.....
8326	Fluxed Bermudez asphalt	45	46	46	46	.....	.....	.....	.....
8615	.....do	115	115	113	115	.....	.....	.....	.....
6293	Blown Gilsonite oil asphalt	140	140	138	138	.....	.....	.....	.....
5104	.....do	60	60	59	60	.....	.....	.....	.....

About the time this work was completed, a second standard needle was obtained from the same source as the one used in the foregoing tests. In order to determine the accuracy with which a number of the new type of needle could be readily made, seven were prepared and checked against both the old and new standard on two distinct types of bituminous material. The results are given in Table IV. Each result is an average of at least three determinations.

TABLE IV.—*Results of a comparative test of new and old standard penetration needles and seven others of the new type*

Needle.	Sample No. 8957 (Gilsonite blown oil asphalt).	Sample No. 8962 (California asphalt).	Needle.	Sample No. 8957 (Gilsonite blown oil asphalt).	Sample No. 8962 (California asphalt).
New standard	94	96	Needle No. 4	92	96
Old standard	91	96	Needle No. 5	91	96
Needle No. 1	89	94	Needle No. 6	91	96
Needle No. 2	90	97	Needle No. 7	90	95
Needle No. 3	91	95			

It will be noted from the above that all seven new needles check very closely with the old standard needle on both samples, and that on sample 8957 they check closer with the old standard than do the two standards with one another. The lack of uniformity in the shape of the two standard needles, the uniformity of the new type of needle, and the relative shapes of the old and new forms of needle are shown in Plate LXXXIII, figure 2, which is a reproduction of an enlarged photograph of the two standard and seven new needles referred to in Table IV.

The following conclusions are offered as a result of the above investigation:

(1) That the No. 2 sewing needle which has heretofore been used for the penetration test can not be taken indiscriminately, but must be carefully selected and standardized.

(2) That there is no recognized established standard with which new needles can be compared, and that it is not feasible to accurately describe the dimensions of a parabola needle.

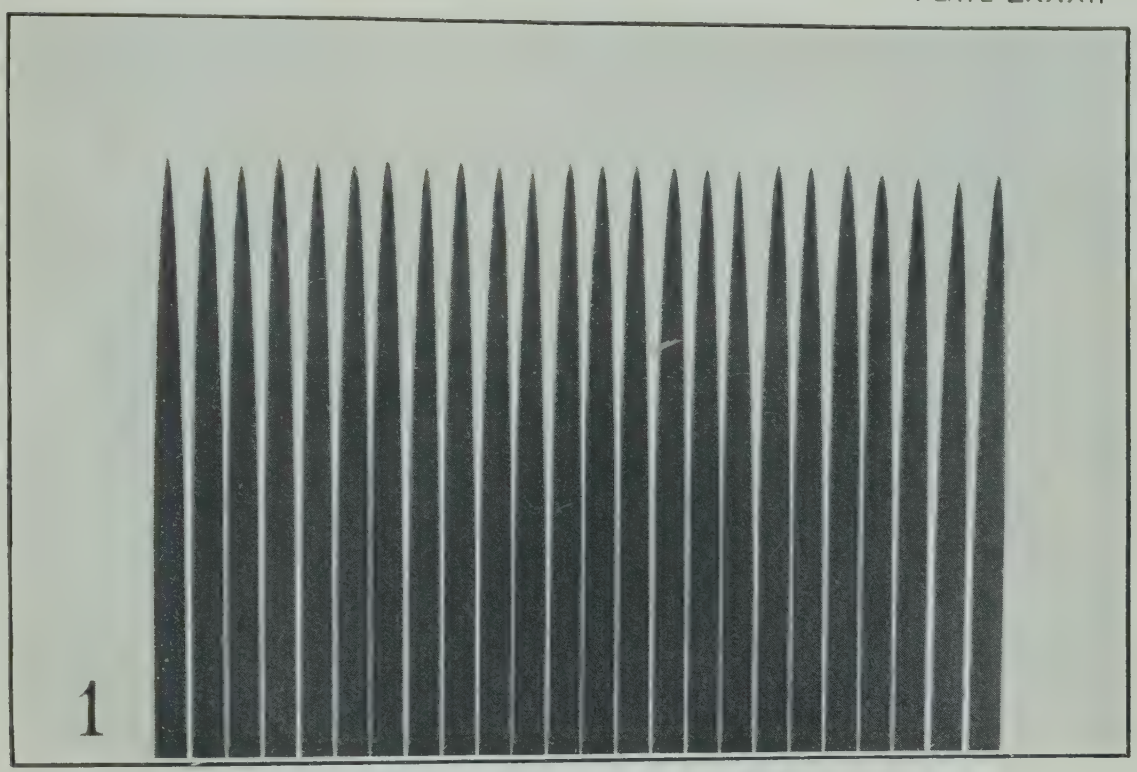
(3) That the so-called standard needles furnished with penetration machines may vary among themselves.

(4) That the writers have designed a needle which gives results in close accord with existing standards and has, moreover, the advantage of being accurately described and easily reproduced.

(5) The needle is made by placing a 2-inch length of 0.041-inch annealed-steel drill rod in the chuck of a high-speed lathe, and by means of a fine sharp file turning the end to a sharp point having a ¼-inch taper. When it has been made as smooth and sharp as possible by this means,

the needle is tempered,[1] then ground to a sharp point with a good stone, after which it is smoothed and polished with emery dust, crocus cloth, and rouge, and finally held carefully on a buffing wheel. The finished needle should be sufficiently smooth and sharp to enter and pass through a piece of ordinary writing paper without sticking or friction. In other words, this new needle must have as sharp a point and smooth a surface as any sewing needle. The important thing is to have the taper straight, beginning ¼ inch from the end, and the needle above the taper exactly 0.04 inch in diameter.

[1] The tempering solution consisted of 5 teacupfuls of common salt, 6 ounces of saltpeter, 12 teaspoonfuls of powdered alum, and 1 teaspoonful of corrosive sublimate dissolved in 10 gallons of water. The needle was tempered by heating carefully to a dull white heat and plunging at once into the tempering solution. It was then lightly cleaned with smooth emery cloth, heated carefully to a point below dull redness, and again plunged into the solution.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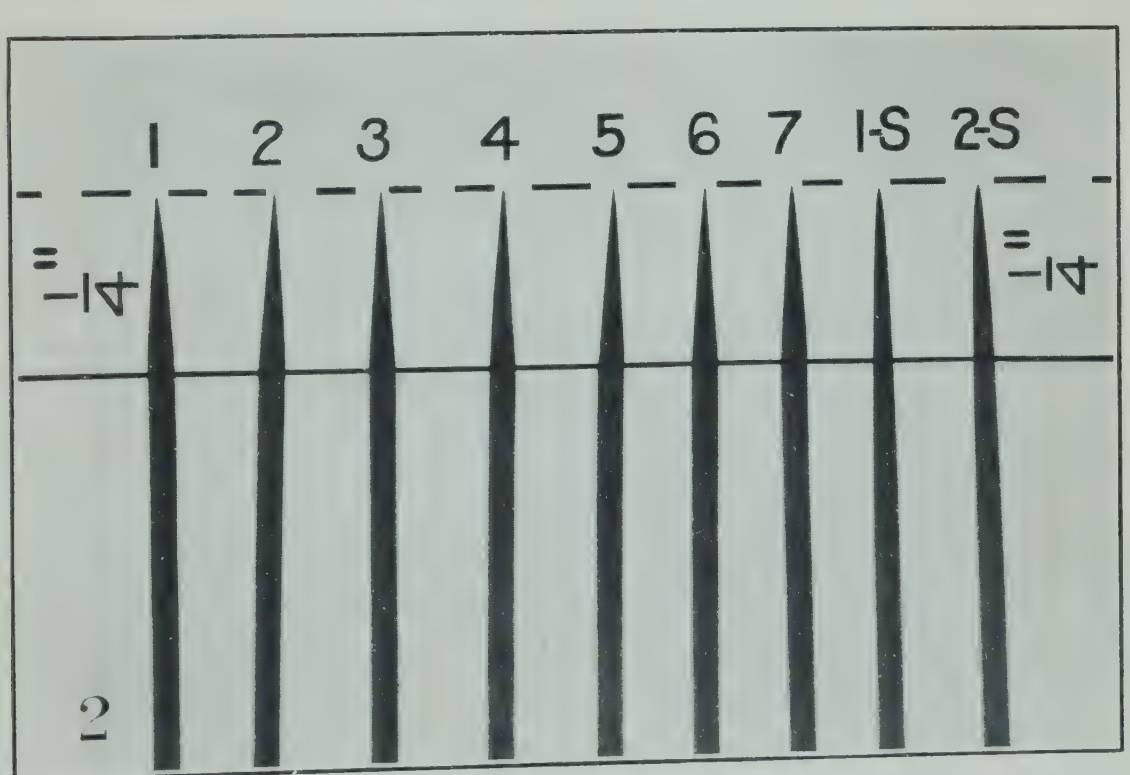
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Journal of Agricultural Research
Vol. V, No. 24

A NEW IRRIGATION WEIR[1]

By V. M. Cone,
Irrigation Engineer, Office of Public Roads and Rural Engineering

INTRODUCTION

The accurate measurement of water delivered to the irrigator has been retarded by lack of information concerning devices adapted to the various conditions of size and grade of canals, and to the sand and silt troubles encountered throughout the West. These conditions are so varied that it is very improbable that any one type of measuring device will be desirable or practicable for all cases. Although the weir is the principal measuring device in use in the West, there are many places where the common types of weirs can not be used, and consequently water users are either making current-meter measurements occasionally or systematically or are doing without any measurement.

Many attempts have been made to devise a weir that would be simple and inexpensive in construction, free from sand troubles, and accurate and simple in operation; but usually what has been gained in one direction has been lost in another.

Weirs with full contractions have been built in many places where sand and silt accumulations have resulted in inaccurate measurements, or constant attention has been required to keep the weir box clean. The first cost of such a weir is rather high and the nuisance and expense of keeping it clean often make it undesirable. In an attempt to overcome these objections many weirs have been built with incomplete contractions which have caused the water to pass through the weir box at a velocity sufficiently high to necessitate the addition of a correction factor to the discharge table, but not high enough to completely prevent the accumulation of sand. It usually occurs that full-contraction-weir tables without correction are used with the modified weirs, and therefore the measurement is not worth much more than the guess of an experienced ditch rider. Damage has resulted from the prevalent belief that the weirs in general carry the stamp of accuracy. Under proper conditions of construction and operation, full-contracted weirs are accurate within a small percentage,[2] but such conditions are not always to be found in the field. In the literature of hydraulics there are practically no records of

[1] The work on which this paper is based was done in the hydraulic laboratory, at Fort Collins, Colo., under a cooperative agreement between the Office of Experiment Stations, United States Department of Agriculture, and the Colorado Agricultural Experiment Station.

[2] Cone, V. M. Flow through weir notches with thin edges and full contractions. *In* Jour. Agr. Research, v. 5, no. 23, p. 1051–1114, 1916.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
Vol. V, No. 24
Mar. 13, 1916
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experiments with weirs having completely suppressed bottom contraction. The idea previously considered seems to have been the suppression of the end contractions in order to secure a simple discharge formula, but such an arrangement of weir box possesses many of the objectionable features of full-contracted weirs. Discharge formulas are infrequently used in the field, tables usually being available, and it therefore seems preferable to have a weir that is practicable and of permanent accuracy rather than to complicate the weir-box conditions in order to simplify the discharge formula. A series of experiments was made in the hydraulic laboratory at Fort Collins, Colo., during the summer of 1914, for the purpose of developing a weir that would be self-cleaning, require a minimum amount of labor and material for construction, measure discharges with an accuracy commensurate with field conditions and irrigation demands, and be easily operated by the ordinary man, which means that only simple readings without any computations would be required to determine the discharge.

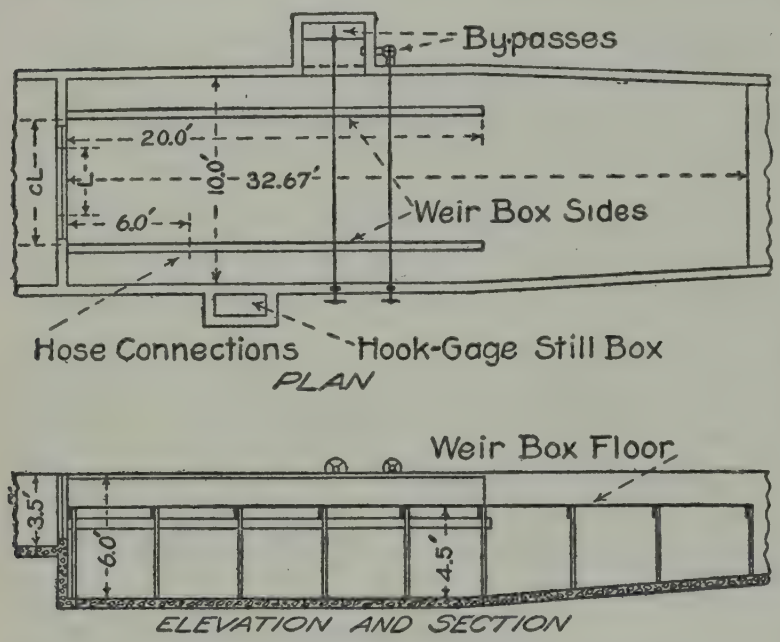

FIG. 1.—Plan, elevation, and section of concrete weir box in the hydraulic laboratory of the Colorado Experiment Station; also arrangement of experimental weir section for Nos. 1 to 6 and 13 to 16, in Table I.

ARRANGEMENT OF APPARATUS FOR EXPERIMENTS WITH NEW TYPE OF WEIR [1]

In the permanent concrete weir box, which is 10 feet wide and 6 feet deep, a wood floor was built of tongue-and-groove lumber (fig. 1). The wood floor was about 4.5 feet above the concrete floor and was water-tight and level throughout. Its length was 20 feet for four sets of experiments, but it was extended to 32.67 feet for all other experiments. The sides of the temporary weir box were made of single widths of boards set in a vertical position, but arranged to be moved to any position or any angle and rigidly fastened to the floor. The several arrangements of the weir box are given in Table I, and figures 1 to 13, inclusive.

[1] For a description of the hydraulic laboratory and equipment, see Cone, V. M., op. cit., and Cone, V. M., Hydraulic laboratory for irrigation investigations, Fort Collins, Colo. *In* Engin. News, v. 70, no. 14, p. 662–665, 5 fig., 1913.

TABLE I.—*Effect of size and shape of weir box on discharge*[1]

SPECIAL TESTS

No.	Length of weir crest.	Width of weir box at crest.	Width of weir box at 20 feet.	Equation of discharge curve.	Fig. No.	Length of floor.	Remarks.
	Feet.					*Feet.*	
1	1	1½ L	1½ L	$Q=4.641\ LH^{1.575}$.....	1	32.67	Sides parallel, no wings.
2	1	2 L	2 L	$Q=3.768\ LH^{1.522}$.....	1	32.67	Do.
3	1	3 L	3 L	$Q=3.441\ LH^{1.498}$.....	1	32.67	Do.
4	1	4 L	4 L	$Q=3.343\ LH^{1.489}$.....	1	32.67	Do.
5	1	5 L	5 L	$Q=3.316\ LH^{1.484}$.....	1	32.67	Do.
6	1	6 L	6 L	$Q=3.274\ LH^{1.479}$.....	1	32.67	Do.
7	1	2 L	3 L	$Q=3.72\ LH^{1.527}$.....	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
8	1	2 L	3 L	$Q=3.69\ LH^{1.506}$.....	3	32.67	Sides extended to sides of concrete box at angle of 45° to axis.
9	1	2 L	3 L	$Q=3.71\ LH^{1.522}$.....	4	32.67	Sides extended to sides of concrete box at angle of 90° to axis.
10	1	2 L	2½ L	$Q=3.73\ LH^{1.516}$.....	4	32.67	Do.
11	1	2 L	2½ L	$Q=3.73\ LH^{1.510}$.....	3	32.67	Sides extended to sides of concrete box at angle of 45° to axis.
12	1.5	2 L	3 L	$Q=3.64\ LH^{1.523}$.....	2	.....	Sides extended at same angle to distance of 32.5 feet from crest.
13	2	1½ L	1½ L	$Q=4.375\ LH^{1.638}$.....	1	32.67	Sides parallel, no wings.
14	2	2 L	2 L	$Q=3.749\ LH^{1.563}$.....	1	32.67	Do.
15	2	2½ L	2½ L	$Q=3.552\ LH^{1.538}$.....	1	32.67	Do.
16	2	3 L	3 L	$Q=3.439\ LH^{1.535}$.....	1	32.67	Do.
17	2	2 L	2 L	$Q=3.749\ LH^{1.546}$.....	5	32.67	Sides parallel, with 45° wings connecting parallel sides 12 feet long, 3 L apart.
18	2	2 L	3 L	$Q=3.63\ LH^{1.549}$.....	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
19	3	2 L	2½ L	$Q=3.640\ LH^{1.560}$.....	6	32.67	Sides extended 12 feet parallel to axis and 2½ L apart.
20	3	2 L	3 L	$Q=3.604\ LH^{1.560}$.....	2	32.67	Sides extended about 5 feet at same angle to sides of concrete box.
21	4	1¼ L	1¼ L	$Q=5.327\ LH^{1.698}$.....	7	20.00	Sides parallel, no wings.
22	4	1½ L	1½ L	$Q=4.105\ LH^{1.599}$.....	7	20.00	Do.
23	4	1½ L	1½ L	$Q=4.053\ LH^{1.634}$.....	2	32.67	Sides parallel, extended to distance of 32.5 feet from crest.
24	4	1¾ L	1¾ L	$Q=3.839\ LH^{1.586}$.....	7	20.00	Sides parallel, no wings.
25	4	2 L	2 L	$Q=3.599\ LH^{1.567}$.....	7	20.00	Do.
26	4	2 L	2 L	$Q=3.590\ LH^{1.580}$.....	2	32.67	Sides parallel, extended to distance of 32.5 feet from crest.
27	4	2 L	2 L	$Q=3.714\ LH^{1.570}$.....	8	32.67	Sides parallel, with 45° wings extending to sides of concrete box.
28	4	2 L	2 L	$Q=3.642\ LH^{1.542}$.....	9	32.67	Sides parallel, with 90° wings extending to sides of concrete box.
29	4	2½ L	2½ L	$Q=3.403\ LH^{1.560}$.....	10	32.67	Full width of concrete box.

STANDARD TESTS

30	1	2 L	2½ L	$Q=3.771\ LH^{1.53}$.....	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
31	1.5	2 L	2½ L	$Q=3.720\ LH^{1.54}$.....	2	32.67	Do.
32	2	2 L	2½ L	$Q=3.690\ LH^{1.54}$.....	2	32.67	Do.
33	3	2 L	2½ L	$Q=3.630\ LH^{1.55}$.....	2	32.67	Do.
34	4	2 L	2½ L	$Q=3.570\ LH^{1.56}$.....	2	32.67	Sides extended at same angle to sides of concrete box.

SPECIAL NOTCH TESTS

	Degrees.	*Feet.*	*Feet.*				
35	90	10	10	$Q=2.541\ H^{2.492}$.....	11	32.67	No sides, channel full width of concrete box.
36	90	3	7	$Q=2.667\ H^{2.521}$.....	12	32.67	Sides extended about 10 feet at same angle to sides of concrete box.
37	90	3	.....	$Q=2.679\ H^{2.517}$.....	13	32.67	Sides 5 feet apart at 10 feet, then extended 12 feet parallel to axis.

[1] Level wood floor placed about 4.5 feet above floor of concrete weir box; angle iron weir crest.

Steel weir plates having rectangular crests and sides made of brass, with nominal crest lengths of 1, 1.5, 2, 3, and 4 feet, were successively attached to the steel frame anchored in the concrete wall. A 2-inch angle iron, dressed and trued, was set flush in the floor section, and by means of bolts the floor section was drawn tightly against the weir plate. The angle iron formed the crest of the weir and it was sufficiently rigid to prevent any trouble due to the possible warping of the floor, and also insured the crests remaining at the same elevation as the floor. The water passed through the weir notch with full lateral expansion and complete aeration of nappe.

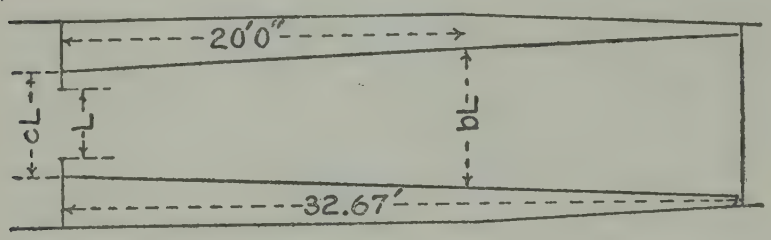

FIG. 2.—Plan of experimental weir box for Nos. 7, 12, 18, 20, and 30 to 34 in Table I.

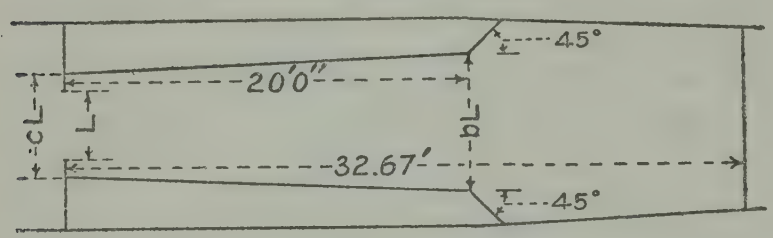

FIG. 3.—Plan of experimental weir box for Nos. 8 and 11, Table I.

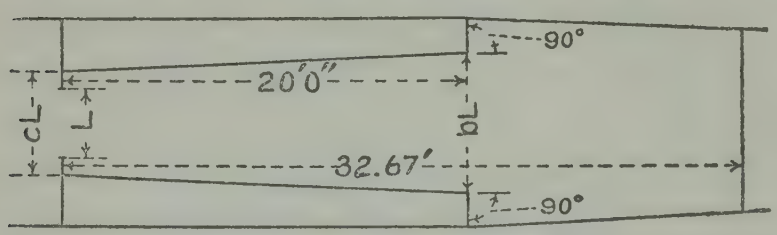

FIG. 4.—Plan of experimental weir box for Nos. 9 and 10, Table I.

The head was determined in the concrete hook-gauge still box which was connected to the weir box by four pieces of ¾-inch hose attached to 1-inch pipe nipples screwed upward through the floor until flush with the surface. The auger holes into which the pipes were screwed were placed near the side of the weir box in a line 6 feet back from the plane of the weir. A second hook gauge was placed in a temporary still box connected by a hose through the side of the weir box near the floor line. This hook gauge was used for check purposes and to determine whether any discrepancies would be introduced by applying the results of the experiments to future installations where the head would be communicated to a still box by pipes through the side of the weir box. The two sets of hook-gauge readings indicated that no error is introduced thereby, provided the pipes are installed at the proper distance from the weir, 6 feet, and in a position normal to the side of the weir box rather than normal to the axis, because the lines of flow are parallel to the side.

In all these experiments the weir discharges were determined volumetrically in the calibrated concrete tanks.

Several series of preliminary experiments were made in order to determine the influence upon the discharge caused by various end contrac-

tion distances, lengths of weir box, contraction wings at entrance of weir box, and angle of sides of weir box. From these data a set of conditions was chosen to be the standard for the new type of weir, for it is obviously necessary that the weir box be definitely standardized in order that the specifications be duplicated in future installations if the formula and tables are to apply. The terms "standard tests" or "standard conditions" will be used to express those conditions which have been taken as the basis of the formula and discharge tables.

The water passes through the weir box with a rather high velocity, but the velocity varies with the head, and the slope of the water surface changes accordingly. The extent of the draw-down curve also varies with the head and length of weir crest and it was therefore necessary to fix the point at which to take the head. Several measurements of draw-down curves resulted in choosing a point 6 feet back from the plane of the weir, which would be away from any considerable influence of draw-down for the weirs used in the experiments, and would not include much of the slope of the water surface.

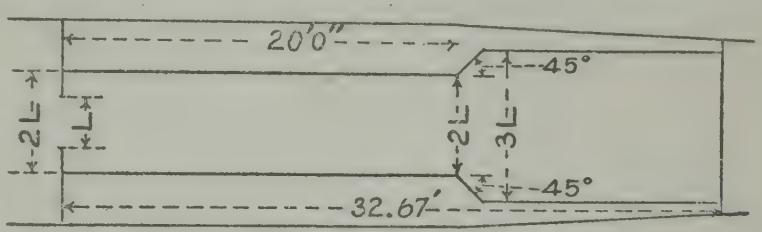

FIG. 5.—Plan of experimental weir box for No. 17, Table I.

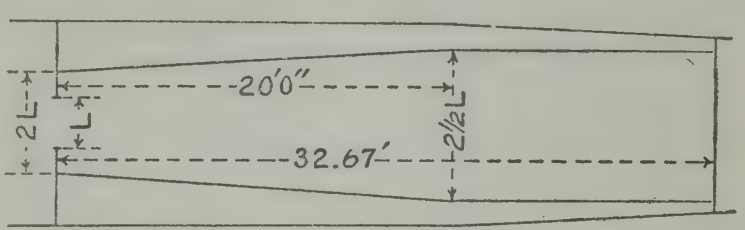

FIG. 6.—Plan of experimental weir box for No. 19, Table I.

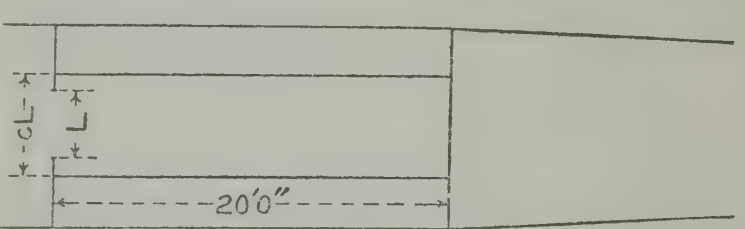

FIG. 7.—Plan of experimental weir box for Nos. 21, 22, 24, and 25, Table I.

A total of 277 experiments were made on this new type of weir, which for want of a better name is called an "irrigation weir," and of this number 101 were preliminary tests and 176 were made under standard conditions.

DEDUCTIONS FROM EXPERIMENTS

The individual equations in simple form for each set of experiments and the conditions under which those experiments were made are given in Table I. The following deductions have been obtained from comparisons of the equations stated in the table, the bottom contractions being entirely suppressed in all cases, but with various arrangements of sides of weir box.

For similar conditions of weir box, the coefficient c decreases as the length of weir crest L increases, and the exponent n increases as the length increases.

As the width of weir box, or end contractions, is increased for any certain length of weir, both c and n decrease. This is probably due to a decrease in the velocity of approach, owing to the increased area of the weir box.

When the sides of the weir box are parallel, the discharge increases as the width of the box is decreased, for all sizes of weirs.

The greatest discharge is obtained when the sides of the weir box are parallel and it decreases as the angle between the sides becomes greater; or, stated in another way, the discharge increases as the sides become more nearly parallel, the width of the box at the weir remaining constant.

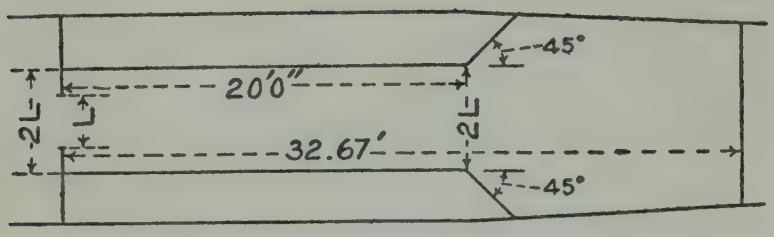

FIG. 8.—Plan of experimental weir box for No. 27, Table I.

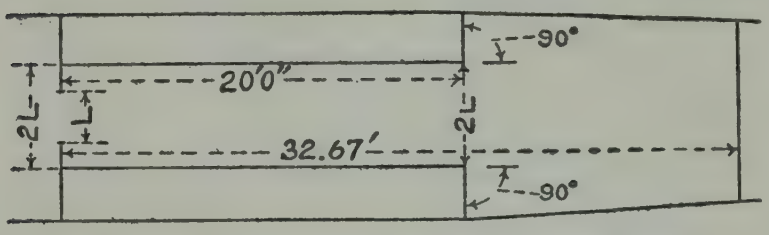

FIG. 9.—Plan of experimental weir box for No. 28, Table I.

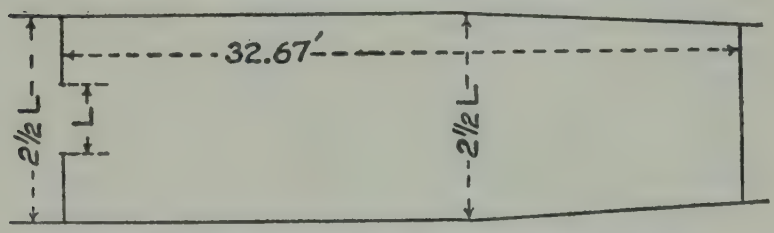

FIG. 10.—Plan of experimental weir box for No. 29, Table I.

When wings placed at the upper end of the weir box to form a junction between the sides of the box and the canal bank are changed from 90° to 45° with the axis of the channel, the discharge is increased for low heads, remains about the same for heads of 0.7 foot, and is decreased for high heads. The percentage of change in discharge due to such a change in the wings is greater when the sides of the weir box are parallel.

The ratio of discharge to length of weir decreases as the length of the weir increases; or, in other words, the discharge over a 4-foot weir is less than four times the discharge over a 1-foot weir, as is shown by the individual standard equations, Nos. 30 to 34, in Table I. This is the reverse of the condition found in rectangular weirs having complete end and bottom contractions and negligible velocity of approach.[1]

If the sides of the weir box are continued parallel from a point 20 feet upstream from the plane of the weir (fig. 6), instead of being continued

[1] Cone, V. M. Flow through weir notches with thin edges and full contractions. *In* Jour. Agr. Research, v. 5, no. 23, p. 1051–1114. 1916.

at the same angle as the other part of the weir box (fig. 2), the discharge will be increased about one-third of 1 per cent for 1-foot head and decreased about 1 per cent for 0.2-foot head, as indicated for the 3-foot weir in Nos. 19 and 33 in Table I.

In addition to the experiments with regular weir notches, three sets of experiments were made with 90° triangular notches having suppressed bottom contraction and different end contractions. The results are represented by Nos. 35, 36, and 37 in Table I. The logarithmic discharge curve for the 90° triangular notch with complete end and bottom contractions is a perfect straight line represented by the equation $Q=2.487h^{2.4805}$. Suppression of the bottom contraction, No. 35 in Table I, resulted in changing the logarithmic discharge curve from a straight line to a curved line, and increased the discharge. An average straight line drawn through the discharge data, represented by the equation $Q=2.541h^{2.492}$, agrees with the experimental data for medium heads, but is about 1 per cent low for high and low heads.

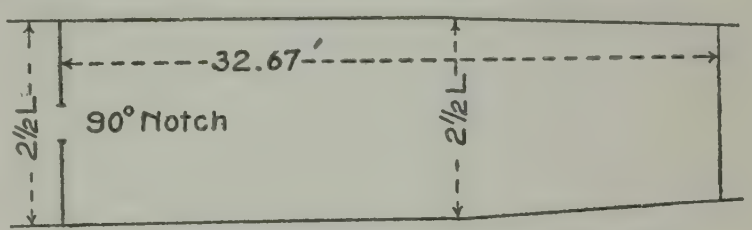

FIG. 11.—Plan of experimental weir box for No. 35, Table I.

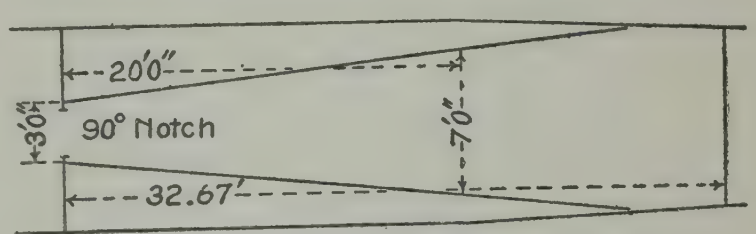

FIG. 12.—Plan of experimental weir box for No. 36, Table I.

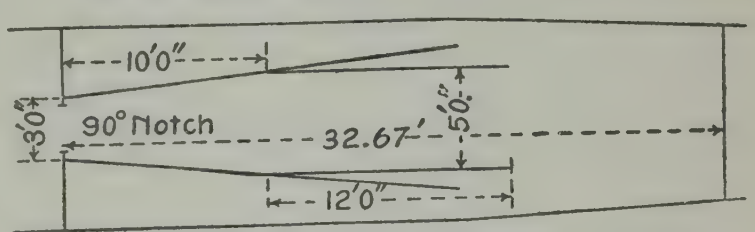

FIG. 13.—Plan of experimental weir box for No. 37, Table I.

The second set of experiments, No. 36 in Table I, also gave a logarithmic plot which was a curved line. The average straight line for these data was about 1 per cent low for heads of 0.3 and 1.3 feet, and about 2 per cent high for heads of approximately 0.8 foot. This indicates the curvature of the discharge plot to be increased by a decrease in end-contraction distances.

The third set of experiments, No. 37 in Table I, was made under conditions which practically amounted to making the weir box 10 feet shorter than in the previous case, having the sides of the carrying channel parallel in both cases, but closer together in this set of experiments. This had little effect upon the discharge in the aggregate, but changed the slope of the discharge curve slightly.

The 90° triangular notch with full contractions is one of the most accurate and reliable measuring devices for small quantities of water.

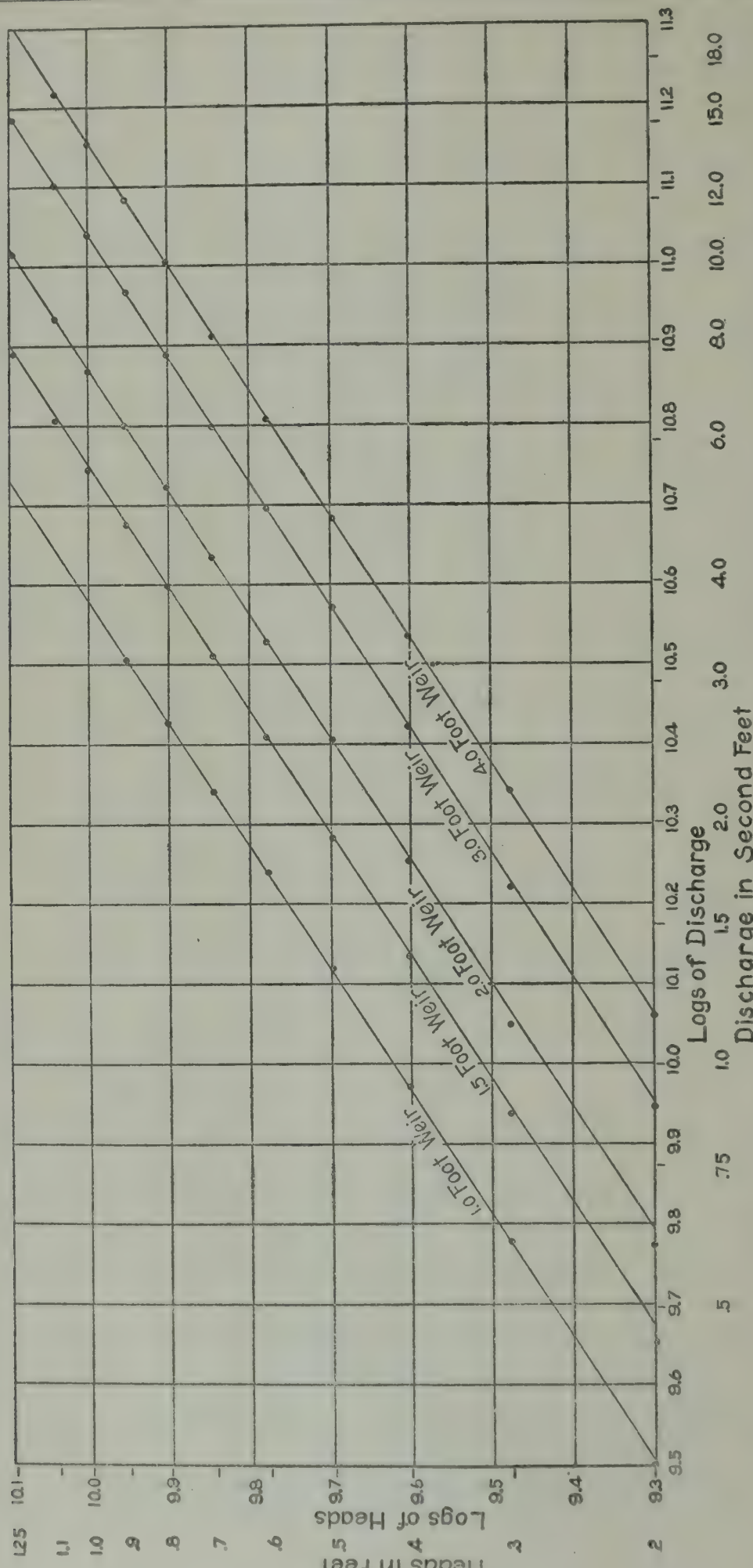

FIG. 14.—Experimental discharge data plotted logarithmically and curves drawn from values computed from standard equation for new irrigation weir.

Suppressing the contractions completely or in part changes the law of discharge through the triangular notch, decreases its accuracy as a practical measuring device, and does not insure the complete removal of sand and silt from the box. It is therefore an open question whether the advantages resulting from suppressed contractions with the triangular notch would not be more than counterbalanced by the inaccuracies introduced. The data are given without recommendation, but may be desirable for use in special cases.

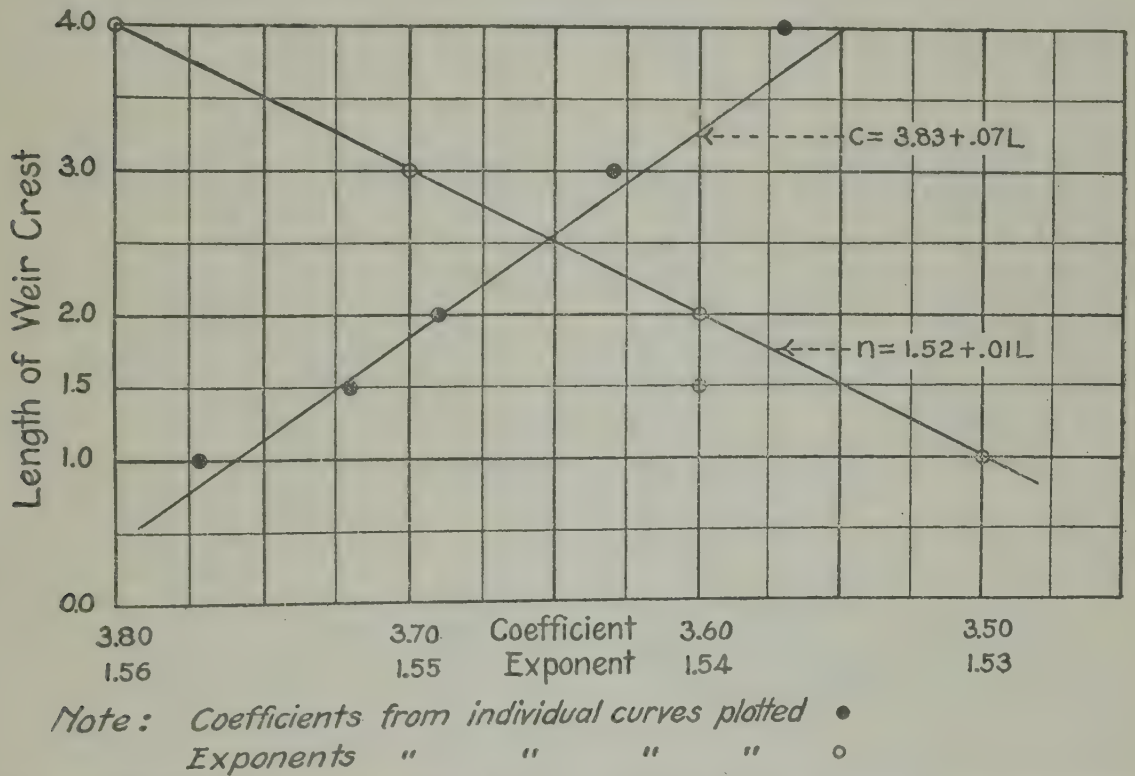

FIG. 15.—Coefficient and exponent values of individual discharge equations plotted against weir length.

DERIVATION OF WEIR FORMULA

The experimental discharge data for the standard weir conditions were plotted logarithmically for weirs having actual crest lengths of 1.0055, 1.5026, 2.0057, 2.9970, and 4.0056 feet, as shown in figure 14. These points do not lie on a straight line. An average straight line drawn through the points will give values too small for medium heads and too large for low and high heads. This characteristic of the curve is the reverse of the curve for rectangular weirs with full contractions, but the suppression of the bottom contraction and partial suppression of the end contraction has tended to straighten the discharge curve.

With full-contraction weirs and quite complete pondage, the head can be accurately determined and there is, therefore, ample reason for using a complicated formula to secure that accuracy of measurement, but the high velocity of water and wave action which occurs in the new irrigation weir preclude the possibility of determining the head accu-

rately enough to warrant any great refinement of the discharge formula. The assumption of straight-line logarithmic formulas is within 1 or 2 per cent of all the discharge data, with the exception of a few high and low heads; and since this is comparable to the accuracy expected under field conditions, such formulas were used to avoid more complicated equations.

The equations of the average straight lines through the plotted points are given in Table I, Nos. 30 to 34, inclusive. The exponent and coefficient values for these individual equations were then plotted against the weir crest lengths, as shown in figure 15. For simplicity the law of the

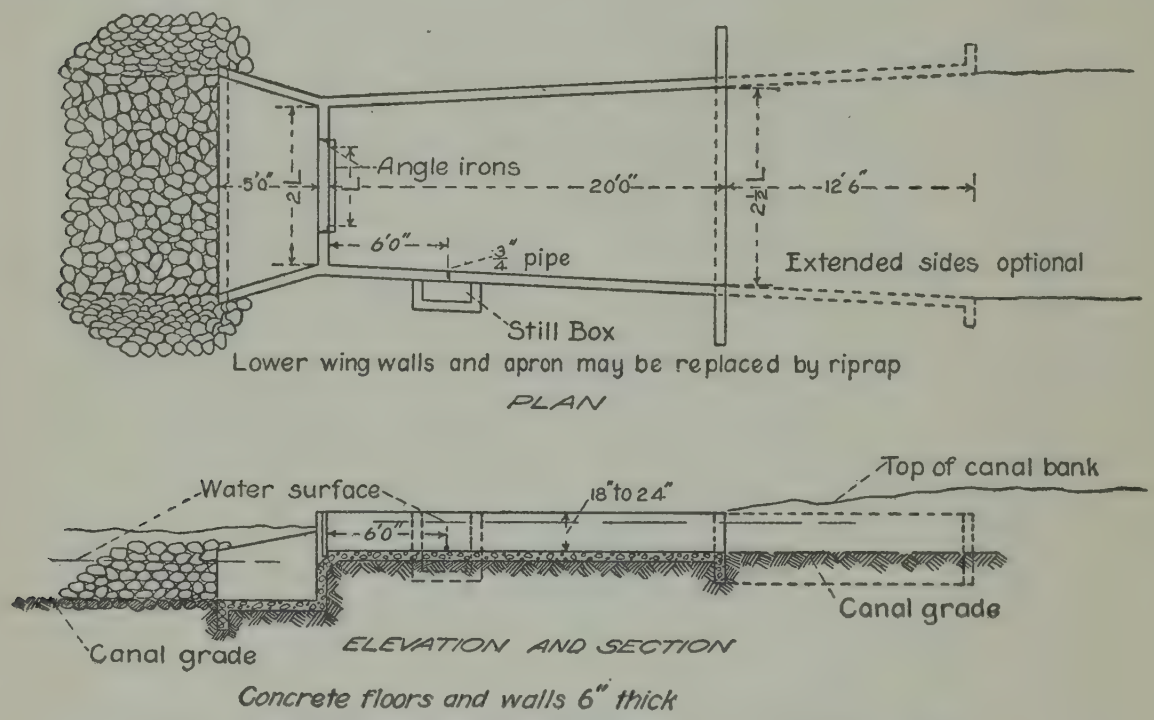

FIG. 16.—Plan, elevation, and section (standard) of new irrigation weir box.

coefficient values was assumed to be represented by the equation $c=(3.83-0.07L)$. The exponents, with the single exception of that for the 1.5-foot weir, fell on the straight line which has the equation $n=(1.52+0.01L)$. By substituting these expressions in the fundamental formula, $Q=cLh^n$, the general formula for the new irrigation weir was obtained

$$Q=(3.83-0.07L)Lh^{(1.52+0.01L)}$$

The straight-line curves drawn in figure 14 for each length of weir represent discharge values computed from the above formula and show graphically the agreement of the formula with the experimental data. The computed discharges are given in Table II.

TABLE II.—*Computed discharges for the new irrigation weirs*

[Computed from the formula $Q=(3.83-0.07\ L)\ Lh^{(1.52+0.01\ L)}$]

Head.	Head.		Length of weir crest.				
			1 foot.	1.5 feet.	2 feet.	3 feet.	4 feet.
Feet.	*Ft.*	*in.*					
0.20	0	2 3/8	0.320	0.472	0.619	0.896	1.15
.21	0	2 1/2	.345	.509	.667	.966	1.24
.22	0	2 5/8	.371	.547	.717	1.04	1.34
.23	0	2 3/4	.397	.586	.768	1.11	1.43
.24	0	2 7/8	.424	.625	.820	1.19	1.53
.25	0	3	.451	.665	.873	1.27	1.63
.26	0	3 1/8	.479	.707	.927	1.34	1.74
.27	0	3 1/4	.507	.749	.982	1.43	1.84
.28	0	3 3/8	.536	.792	1.04	1.51	1.95
.29	0	3 1/2	.566	.836	1.10	1.59	2.06
.30	0	3 5/8	.596	.880	1.16	1.68	2.17
.31	0	3 3/4	.626	.926	1.22	1.77	2.28
.32	0	3 13/16	.658	.972	1.28	1.86	2.40
.33	0	3 15/16	.690	1.02	1.34	1.95	2.52
.34	0	4 1/16	.722	1.07	1.40	2.04	2.64
.35	0	4 3/16	.754	1.12	1.47	2.13	2.76
.36	0	4 5/16	.788	1.16	1.53	2.23	2.88
.37	0	4 7/16	.822	1.21	1.60	2.33	3.01
.38	0	4 9/16	.856	1.27	1.66	2.42	3.14
.39	0	4 11/16	.890	1.32	1.73	2.52	3.27
.40	0	4 13/16	.925	1.37	1.80	2.62	3.40
.41	0	4 15/16	.961	1.42	1.87	2.73	3.53
.42	0	5 1/16	.997	1.48	1.94	2.83	3.67
.43	0	5 3/16	1.03	1.53	2.01	2.94	3.81
.44	0	5 1/4	1.07	1.58	2.08	3.04	3.94
.45	0	5 3/8	1.11	1.64	2.16	3.15	4.09
.46	0	5 1/2	1.15	1.70	2.23	3.26	4.23
.47	0	5 5/8	1.18	1.75	2.31	3.37	4.37
.48	0	5 3/4	1.22	1.81	2.38	3.48	4.52
.49	0	5 7/8	1.26	1.87	2.46	3.59	4.67
.50	0	6	1.30	1.93	2.54	3.71	4.82
.51	0	6 1/8	1.34	1.99	2.62	3.82	4.97
.52	0	6 1/4	1.38	2.05	2.70	3.94	5.12
.53	0	6 3/8	1.42	2.11	2.78	4.06	5.27
.54	0	6 1/2	1.46	2.17	2.86	4.18	5.43
.55	0	6 5/8	1.51	2.23	2.94	4.30	5.59
.56	0	6 3/4	1.55	2.29	3.02	4.42	5.75
.57	0	6 13/16	1.59	2.36	3.11	4.54	5.91
.58	0	6 15/16	1.63	2.42	3.19	4.67	6.07
.59	0	7 1/16	1.68	2.49	3.27	4.79	6.23
.60	0	7 3/16	1.72	2.55	3.36	4.92	6.40
.61	0	7 5/16	1.76	2.62	3.45	5.05	6.57
.62	0	7 7/16	1.81	2.68	3.53	5.18	6.74
.63	0	7 9/16	1.85	2.75	3.62	5.31	6.91
.64	0	7 11/16	1.90	2.82	3.71	5.44	7.08
.65	0	7 13/16	1.95	2.88	3.80	5.57	7.25
.66	0	7 15/16	1.99	2.95	3.89	5.70	7.43
.67	0	8 1/16	2.04	3.02	3.98	5.84	7.60
.68	0	8 3/16	2.08	3.09	4.08	5.97	7.78
.69	0	8 1/4	2.13	3.16	4.17	6.11	7.96

TABLE II.—*Computed discharges for the new irrigation weirs*—Continued

Head.	Head.		Length of weir crest.				
			1 foot.	1.5 feet.	2 feet.	3 feet.	4 feet.
Feet.	*Ft.*	*in.*					
0.70	0	8 3/8	2.18	3.23	4.26	6.25	8.14
.71	0	8 1/2	2.23	3.30	4.35	6.39	8.32
.72	0	8 5/8	2.27	3.37	4.45	6.53	8.50
.73	0	8 3/4	2.32	3.45	4.55	6.67	8.69
.74	0	8 7/8	2.37	3.52	4.64	6.81	8.88
.75	0	9	2.42	3.59	4.74	6.95	9.06
.76	0	9 1/8	2.47	3.67	4.84	7.10	9.25
.77	0	9 1/4	2.52	3.74	4.94	7.24	9.44
.78	0	9 3/8	2.57	3.82	5.03	7.39	9.64
.79	0	9 1/2	2.62	3.89	5.13	7.54	9.83
.80	0	9 5/8	2.67	3.97	5.23	7.68	10.02
.81	0	9 3/4	2.72	4.04	5.34	7.83	10.22
.82	0	9 13/16	2.78	4.12	5.44	7.98	10.42
.83	0	9 15/16	2.83	4.20	5.54	8.14	10.62
.84	0	10 1/16	2.88	4.28	5.64	8.29	10.82
.85	0	10 3/16	2.93	4.35	5.75	8.44	11.02
.86	0	10 5/16	2.99	4.43	5.85	8.60	11.22
.87	0	10 7/16	3.04	4.51	5.95	8.75	11.43
.88	0	10 9/16	3.09	4.59	6.06	8.91	11.63
.89	0	10 11/16	3.15	4.67	6.17	9.07	11.84
.90	0	10 13/16	3.20	4.75	6.27	9.22	12.05
.91	0	10 15/16	3.25	4.83	6.38	9.38	12.26
.92	0	11 1/16	3.31	4.92	6.49	9.54	12.47
.93	0	11 3/16	3.36	5.00	6.60	9.70	12.68
.94	0	11 1/4	3.42	5.08	6.71	9.87	12.89
.95	0	11 3/8	3.48	5.16	6.82	10.03	13.11
.96	0	11 1/2	3.53	5.25	6.93	10.19	13.32
.97	0	11 5/8	3.59	5.33	7.04	10.36	13.54
.98	0	11 3/4	3.65	5.42	7.15	10.53	13.76
.99	0	11 7/8	3.70	5.50	7.27	10.69	13.98
1.00	1	0	3.76	5.59	7.38	10.86	14.20
1.01	1	0 1/8	3.82	5.67	7.49	11.03	14.42
1.02	1	0 1/4	3.88	5.76	7.61	11.20	14.64
1.03	1	0 3/8	3.93	5.85	7.72	11.37	14.87
1.04	1	0 1/2	3.99	5.93	7.84	11.54	15.10
1.05	1	0 5/8	4.05	6.02	7.96	11.71	15.32
1.06	1	0 3/4	4.11	6.11	8.07	11.89	15.55
1.07	1	0 13/16	4.17	6.20	8.19	12.06	15.78
1.08	1	0 15/16	4.23	6.29	8.31	12.24	16.01
1.09	1	1 1/16	4.29	6.38	8.43	12.41	16.24
1.10	1	1 3/16	4.35	6.47	8.55	12.59	16.48
1.11	1	1 5/16	4.41	6.56	8.66	12.77	16.71
1.12	1	1 7/16	4.47	6.65	8.79	12.94	16.95
1.13	1	1 9/16	4.53	6.74	8.91	13.12	17.18
1.14	1	1 11/16	4.59	6.83	9.03	13.30	17.42
1.15	1	1 13/16	4.66	6.92	9.15	13.49	17.66
1.16	1	1 15/16	4.72	7.02	9.28	13.67	17.90
1.17	1	2 1/16	4.78	7.11	9.40	13.85	18.14
1.18	1	2 3/16	4.84	7.20	9.52	14.04	18.38
1.19	1	2 1/4	4.91	7.30	9.65	14.22	18.63

TABLE II.—*Computed discharges for the new irrigation weirs*—Continued

Head.	Head.	Length of weir crest.				
		1 foot.	1.5 feet.	2 feet.	3 feet.	4 feet.
Feet.	*Ft. in.*					
1.20	1 2⅜	4.97	7.39	9.77	14.41	18.87
1.21	1 2½	5.03	7.49	9.90	14.59	19.12
1.22	1 2⅝	5.10	7.58	10.02	14.78	19.36
1.23	1 2¾	5.16	7.68	10.15	14.97	19.61
1.24	1 2⅞	5.23	7.77	10.28	15.16	19.86
1.25	1 3	5.29	7.87	10.41	15.35	20.11

Table III shows the differences between the discharges computed from the formula and those obtained by experiment, these differences being expressed in cubic feet per second and in percentages. The formula agrees with the experimental data within a maximum amount of 4.8 per cent for an individual point, but this discrepancy is no doubt due partly to experimental inaccuracy and partly to the assumption of a straight-line formula. Medium heads give values for discharges that agree within 1 per cent, but the high and low heads will have a somewhat greater error. The formula agrees with the average straight lines drawn through the experimental data within a maximum error of 1 per cent. The error is greatest with the small weirs, decreases as the length of the weir increases, and for a length of 4 feet the error is quite small. Although the formula is derived from experiments with weirs having a maximum length of 4 feet it seems probable that the formula will be even closer for weirs with greater crest lengths.

TABLE III.—*Difference between discharges computed from the formula* $Q=[3.83-0.07L]LH^{(1.52+0.01L)}$ *and those obtained by experiment, for the new type of weir*

1-FOOT WEIR

Head.	Observed Q corrected true for length.	Computed Q.	Difference in Q.	Percentage of difference.[1]
Feet.				
0.200	0.314	0.320	+0.006	+1.94
.300	.595	.596	+ .001	+ .17
.400	.935	.925	+ .010	+1.07
.500	1.299	1.302	+ .003	+ .20
.599	1.727	1.716	− .011	− .60
.699	2.183	2.174	− .009	− .40
.800	2.661	2.673	+ .012	+ .50
.895	3.113	3.173	+ .060	+1.92

[1] Percentage of difference between discharge obtained by computations from the formula $Q=[3.83-0.07L]LH^{(1.52+0.01L)}$ and by experiment, the bases of comparison being the experimental data.

TABLE III.—*Difference between discharges computed from the formula* $Q=[3.83-0.07L]LH^{(1.52+0.01L)}$ *and those obtained by experiment, for the new type of weir*—Continued

1.5-FOOT WEIR

Head.	Observed Q corrected true for length.	Computed Q.	Difference in Q.	Percentage of difference.
Feet.				
0.199	0.448	0.469	+0.021	+4.69
.299	.865	.876	+ .011	+1.30
.400	1.360	1.369	+ .009	+ .66
.497	1.907	1.910	+ .003	+ .16
.600	2.560	2.551	− .009	− .35
.700	3.227	3.232	+ .005	+ .15
.800	3.956	3.967	+ .011	+ .28
.900	4.728	4.753	+ .025	+ .53
.998	5.521	5.570	+ .049	+ .89
1.099	6.378	6.459	+ .081	+1.27
1.250	7.727	7.870	+ .143	+1.85

2-FOOT WEIR

0.200	0.590	0.619	+0.029	+4.80
.300	1.116	1.156	+ .040	+3.58
.400	1.784	1.800	+ .016	+ .90
.500	2.536	2.538	+ .002	+ .08
.600	3.358	3.361	+ .003	+ .09
.700	4.288	4.261	− .027	− .63
.800	5.179	5.234	+ .055	+1.06
.900	6.279	6.274	− .005	− .08
1.000	7.358	7.380	+ .022	+ .30
1.100	8.540	8.547	+ .007	+ .08
1.250	10.335	10.406	+ .071	+ .69

3-FOOT WEIR

0.200	0.884	0.896	+0.012	+1.36
.300	1.663	1.680	+ .017	+1.02
.396	2.583	2.584	+ .001	+ .04
.501	3.720	3.720	.000	.00
.598	4.938	4.895	− .043	− .85
.700	6.297	6.248	− .049	− .78
.800	7.754	7.684	− .070	− .90
.900	9.287	9.223	− .064	− .69
1.001	10.948	10.877	− .071	− .65
1.100	12.638	12.589	− .049	− .39
1.250	15.331	15.347	+ .016	+ .10

TABLE III.—*Difference between discharges computed from the formula* $Q=[3.83-0.07L]LH^{(1.52+0.01L)}$ *and those obtained by experiment, for the new type of weir*—Continued

4-FOOT WEIR

Head.	Observed Q corrected true for length.	Computed Q.	Difference in Q.	Percentage of difference.
Feet.				
0.200	1.148	1.153	+0.005	+0.44
.301	2.188	2.182	− .006	− .27
.399	3.417	3.387	− .030	− .88
.500	4.806	4.817	+ .011	+ .23
.601	6.427	6.417	− .010	− .16
.700	8.158	8.141	− .017	− .21
.799	10.045	10.006	− .039	− .39
.900	12.081	12.047	− .034	− .28
1.000	14.194	14.200	+ .006	+ .04
1.100	16.426	16.476	+ .050	+ .30

SPECIFICATIONS FOR CONSTRUCTION AND USE OF THE NEW IRRIGATION WEIR

A plan and elevation of the standard weir is shown in figure 16. The weir notch is rectangular in form, with sharp crest and sides. The floor of the weir box must be level with the crest, and it is therefore convenient to use an angle iron for the crest, embedding one face of the angle until flush with the surface of the floor, the other face of the angle extending downward. The sides of the weir notch may also be made of angle iron placed in a vertical position, with one end extending below the crest and one face of the angle against the angle-iron crest. The angle can then be attached to the weir bulkhead through holes placed in the other face. This arrangement is durable and inexpensive and will meet the requirement of sharp crest and full lateral expansion for the escaping stream of water. The grade of the canal downstream from the weir must be low enough to give free fall and complete aeration to the nappe.

The floor of the weir box must be level throughout, and there must be no sudden or decided differences in elevation between the floor and the grade of the channel of approach. The weir box must be placed in the center of the ditch, so the axial line of the box corresponds with the axial line of the canal, in order that the water may enter the weir box in straight lines. The width of the weir box must be twice the length of the weir crest ($2\ L$) at the plane of the weir, and two and a half times the length of the weir crest ($2\frac{1}{2}\ L$) at a distance of 20 feet upstream from the plane of the weir. The standard tests were made with a weir box 32.5 feet long, except for the 4-foot weir, No. 34, Table I, and the sides were extended at the angle indicated above. However, from Table I, Nos. 7, 8, and 9,

and 10, 11, and 30, it will be seen that for the 1-foot weir at least the discharge through a box 32.5 feet long with sides set to the standard dimensions is within 1 per cent of the discharge obtained by placing 90° wings at the end of a similar box 20 feet long. The use of 45° wings will cause an error of about 2½ per cent. Therefore the weir box for the new irrigation weir should be made with sides spaced 2 L at the plane of the weir and 2½ L at 20 feet upstream from the weir, with the sides continuing at this angle until they meet the banks of the ditch or canal; or the box should be only 20 feet long with wings to connect the sides of the box with the canal banks, and these wings should form an angle of 90° with the axis of the weir box. The 90° wings (fig. 2) give a discharge about 1 per cent greater than with the extended sides (fig. 4) for a head of 0.2 foot and about 1 per cent less for a head of 1 foot.

Extending the sides of the weir box until they are the full size of the canal will give more accurate results, but this accuracy may not be required, and the saving in cost of construction due to the shorter length of the weir box with wings may be more desirable than the 1 per cent of accuracy in measuring the water. Unless the canal bottom is easily eroded or scoured, it would not be necessary to extend the floor of the weir box beyond 20 feet, even if the sides of the box are extended.

The comparatively high velocity of the water flowing through the weir box causes a wave action and generally disturbed condition of the water surface, which makes it quite impossible to determine the head h in the open weir box. Any stilling device placed in the weir would interfere with the action of the weir, and it is therefore necessary that a still box be placed outside the weir box and connected through the side of the weir box with one or more 1-inch pipes located 6 feet from the plane of the weir. The pipe should be placed near the floor of the weir box to insure its being submerged for low heads, and care must be used to place the pipe normal to the side of the weir box, and not normal to the axis of the box. If the pipe is pointed downstream the velocity of the water in the weir box will cause a suction action which will make the water surface in the still box lower than that in the weir box. If the pipe is pointed upstream, there will be a velocity head added to the actual water level in the weir box, and the water in the still box will be higher than that in the weir box. Although no sand or silt will accumulate in the weir box, regardless of the amount carried by the stream, silt may be deposited in the still box and clog the connection pipe unless it is cleaned regularly. By making a deep still box, space will be provided for such silt accumulation and therefore less frequent cleaning will be required. The still box should have inside dimensions of at least 1 foot by 1½ or 2 feet, with such depth as is necessary. The head in the still box may be determined by means of a scale, a hook gauge, or an automatic registering gauge.

The new irrigation weir may be constructed of lumber, but the design is such that it may be easily constructed of concrete. There would be

no difficult form work required for the concrete, and it would make an inexpensive, durable, and satisfactory measuring device, especially if the angle-iron sides and crest of notch were used in connection with the concrete box.

ADVANTAGES OF THE NEW IRRIGATION WEIR

(1) The new irrigation weir is self-cleaning. The increasing velocity of the water from the time it enters the weir box until it passes through the weir notch prevents the deposit of sand and silt. Floating materials are also carried through the weir.

(2) No lowering of the canal grade or building up of the banks is required for the construction of the weir box. The weir box has only one-fourth the depth and a less width than is required for a full-contraction weir. Less excavation and less materials are needed in the construction, and the cost of the weir is therefore greatly decreased.

(3) It may be installed by the farmer without expert assistance and with the tools ordinarily at hand. Its operation does not require special training.

(4) Its accuracy is consistent with practical demands and will remain constant.

(5) It can not be easily tampered with or accidentally injured so as to alter its discharge.

(6) There are no working parts which require attention for proper operation. There is practically no upkeep expense if the weir is well constructed of durable materials.

(7) When the discharge tables are used, no computations are required, because the effect of velocity of approach is incorporated in the tables. The weir discharge is expressed in cubic feet per second, which may be converted into any units desired. An automatic recording gauge used in connection with this weir will give a record of the quantity of water discharged at all times, and the aggregate discharge can be computed from the record if desired.

(8) It is not patented, and the entire cost of the weir is for materials and the labor of construction.

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JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., MARCH 20, 1916 NO. 25

INHERITANCE OF FERTILITY IN SWINE[1]

[PRELIMINARY PAPER]

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INTRODUCTION

Mendelian inheritance applies almost without exception to the transmission of qualitative characters. Quantitative traits, on the other hand, are susceptible only to a generalized treatment from this viewpoint, and few investigators have attacked the problem. Size inheritance in animals has been dealt with by Castle and Phillips (2)[2], Goldschmidt (7), MacDowell (10), Phillips (19, 20), and Punnett and Bailey (21), while Detlefsen (3) has treated the inheritance of certain skeletal characters. Pearl, (15) discovered an arbitrary division point of 30 eggs in the winter laying period of hens, for which inheritance apparently depends on two factors, one of which follows an ordinary Mendelian, and the other a sex-linked scheme. These determiners provide the nearest to units of inheritance that have yet been isolated in quantitative studies.

Because of the fact that fecundity deviates only by discrete units, the litter size in swine provides peculiarly favorable material for studying quantitative inheritance. An analysis of this material has already been attempted from the biometric viewpoint. Rommel and Phillips (24) correlated the size of litters in which dams and daughters were farrowed and found a correlation coefficient of 0.0601 ± 0.0086. They conclude from this result that there is an actual positive correlation between the size of litters of two successive generations, believing that size of litter is a character transmitted from mother to daughter. They recognize the smallness of the coefficient, but believe the indications of inheritance are large enough to provide a basis for selection. In studying fertility inheritance Pearson and Lee (18) obtained practically similar coefficients with the human race and the thoroughbred horse. The range of correlation was 0.0418 to 0.213; hence, they conclude that fertility is certainly and markedly inherited.

[1] Paper No. 1 from the Laboratory of Animal Technology, Kansas Experiment Station.

[2] Reference is made by number to "Literature cited," p. 1159–1160.

Rommel and Phillips (24) studied the inheritance only through the female line, taking no account of a possible influence of the male. George (6) correlated the size of litter with that of the paternal and maternal grandams, respectively. Only 296 litters were involved in his populations; hence, his probable errors were large. But in the dam and daughter comparisons he approximated very closely the result obtained by Rommel and Phillips. His four coefficients follow:

Daughter and dam	0.0615±0.0390
Dam and grandam	.1147± .0343
Daughter and maternal grandam	.0025± .0392
Daughter and paternal grandam	.0508± .0392

None of these correlations are three times as large as their probable error; hence, none are really significant.

Simpson (25) approached the problem from a Mendelian standpoint by crossing a wild German Schwarzwald boar to a young Tamworth sow. The Schwarzwald normally averages 4 pigs to the litter, the Tamworth about 11. The particular sow used was farrowed in a litter of 12 pigs, and to the stint of the wild boar farrowed 9 pigs. In the F_1 generation three females were bred, one to a litter mate and the other two to sires unnamed. The first sow produced 4 pigs, the others 4 and 6, respectively, all in their first litters. The sow producing the 6-pig brood was later served by a pure Schwarzwald boar and farrowed 7 pigs, being apparently constant for that degree of fertility. One of the sows from the brood of 6 gave birth to 12 pigs when mated to a pure Tamworth male. The evidence for a segregation of fecundity factors seems fairly clear, although the numbers are small.

NONGENETIC FACTORS AFFECTING FERTILITY

External factors play a great part in the realization of the inborn hereditary capacity for reproduction. Marshall (12, 13) discusses at length the relation between season and productivity, while the sterility of wild animals in captivity or of domestic animals transferred to vastly different altitudes is proverbial. Marshall and Evvard (4, 5) have both studied the effect of "flushing" in sheep, and Evvard has conducted some very exhaustive investigations into the relation of the various compounds of nutrition to litter size in swine. Using the rate of gain at breeding time in gilts [1] as an indication of the state of nutrition, Evvard has found as much as an average difference of two pigs per litter in favor of the best gainers in each experimental lot, when compared with the poorest gainers. Protein added to a nitrogen-deficient ration (corn alone) produced a marked rise in the fertility of gilts and a medium rise in the fertility of older sows.

Many stockmen believe that overfatness diminishes fecundity. There may be both a physical obstruction of the reproductive organs due to

[1] A gilt is a young sow intended for breeding purposes. The term is usually applied only until the first litter is produced, although it is sometimes extended throughout the suckling period.

fat and an adipose degeneration of the sex glands. Whether these are really causes of decreased fertility is doubtful, since the best evidence shows them to be symptoms of reproductive derangement.

Overfatness occurs frequently as a result of disturbances in the metabolism, due to loss of secretion from several of the ductless glands, the sex gland being here included. Castrating or spaying are known to promote obesity; hence, it is quite reasonable to assume that if testicular or ovarian derangement first occurs, then fat deposition will follow. Overfatness would thus merely indicate and not initiate reduced fecundity.

Market hog raisers usually believe that pure-bred hogs are deteriorating in prolificacy, in line with the common idea that inbreeding ultimately results in barrenness. Bitting, in 1898 (1), investigated the average size of the first 200 litters and the last 200 litters recorded at that time in the herdbooks of the Berkshire, Ohio Poland-China, Standard Poland-China, and Improved Chester White registry associations and found that during the period in which registration had taken place the Berkshires had decreased 0.19 pig per litter, the Poland-China had increased 0.225 pig, and the Chester White had increased 0.1 pig. Rommel (22) investigated the same point for a period of 20 years in books of the American and Ohio Poland-China associations, comparing the average size of litter for the first 5 years with the average for the last 5. The increase was 0.62 pig per litter among the American Poland-Chinas and 0.43 pig per litter in the Ohio strain. A similar study by Rommel (22) on the Duroc-Jersey covering over 15 years showed an increase of 0.57 pig. The changes which have occurred here are manifestly opposed to the idea that purity of blood lines diminishes fertility. On the other hand, the purity of blood can not be credited with the increase, since a constant selection for large litters has taken place, although an increased homozygosis for prolificacy might come about gradually with years of such mass selection as ordinary stock breeding involves.

Hammond (8) has shown that ova may be lost either before or after fertilization; and, still more important, he has discovered that a relatively high percentage may atrophy during the earlier stages of embryonic growth. Lewis (9) indicated that there may be morphological interferences with reproduction, so that fertility may be decreased. He found that the sperm cells of the boar are practically all dead after being in the uterus for 48 hours, which would, of course, result in a reduced fertility. Lewis's results on the viability of sperm differ from those of Dührssen (11), who observed living sperms in the Fallopian tubes of a woman patient three weeks after copulation had taken place. The importance of this question is probably confined to individual cases.

Certain relatively extraneous characters are popularly supposed to be correlated with high fertility. Many farmers believe that "big type" or "cold blooded" hogs farrow larger litters than "hot blooded," or

that "Spotted Poland-Chinas" are far more fecund than ordinary strains. Swine judges commonly consider long-bodied sows more prolific than their chubbier mates. A comparison of 1,000 litters of "large type" Poland-Chinas with 1,100 litters of "small type" showed no significant difference in fertility. The mean for the "large type" was 7.854 ± 0.0456, and for the "small type" was 7.896 ± 0.0436. Furthermore, the standard deviation of the two groups was almost exactly the same, being 2.142 ± 0.0323 for the former and 2.146 ± 0.0309 for the latter. The writers have never seen more than isolated instances brought forward to confirm the popular ideas on this subject and feel that the bulk of such beliefs have resulted from mere advertising schemes.

Breed certainly has its influence. Bitting (1) has averaged the litter sizes for 400 Berkshires, 1,086 Poland-Chinas, and 600 Chester Whites, with the following results:

Berkshire	8.22 pigs per litter.
Poland-China	7.45 pigs per litter.
Chester White	8.96 pigs per litter.

Surface (26) computed the means and standard deviations in the 54,515 litters of Poland-Chinas and the 21,652 litters of Duroc-Jerseys studied by Rommel (22). His constants follow:

	Mean.	Standard deviation.
Poland-China	7.435 ± 0.01	2.038 ± 0.013
Duroc-Jersey	$9.337 \pm .021$	$2.427 \pm .016$

The large numbers here involved undoubtedly prove that real breed differences in fertility exist.

Pearl (15, 16) found the number of mammæ to be correlated positively with the number at a birth when different species are compared, but the coefficient is very low within the species. Parker and Bullard (14) correlated the same characters in 1,000 litters of swine and obtained a coefficient of 0.0035 ± 0.0124. The senior author[1] treated the same point in 170 litters of which he had made genetic studies and obtained a coefficient of -0.0059 ± 0.0517.

These figures certainly demonstrate that the number of mammæ in swine is not related to fertility; in fact, nothing so far discussed presents reliable external characters on which fertility selections can be made. Apparently fecundity has as profound a genetic as physiologic basis.

VALUE OF HERDBOOK DATA

There is now on record an immense mass of data relating to fertility inheritance in swine, in the volumes of the different breed registry associations. In addition to the name and number of the animal, its parents, breeder, etc., the size of litter in which it was farrowed is usually stated.

[1] Unpublished data.

This furnishes opportunity to link together any desired number of generations.

In treating such data, the degree of confidence which can be placed in the figure for litter size must be considered. Its accuracy depends on the carefulness and honesty of the breeder, the accuracy of the clerks in the registry office, and the freedom from typographical errors in the printing of the volume. The matter of personal integrity can be accepted to a high degree, for fortunately the majority of breeders are quite reliable. Whenever falsification wittingly occurs, the tendency is to raise the number per litter; but, owing to the publicity involved in pure-bred breeding as well as the personality invested in breeding animals due to the registry systems, it is doubtful if litter sizes are ever exaggerated by more than one or two pigs.

Investigations in color discrepancies, mistakes in parentage, etc., have shown that about 2 per cent of errors are involved in the work of registry-office clerks and in printing. Some associations are more careful than others, but, of course, none are absolutely free from errors. Unfortunately swine books show a relatively greater number of mistakes than do those published by breeders of some of the other classes of live stock.

However, assuming, as has been done, that the bulk of the records can be accepted, there still remains a question as to their genetic value. It is doubtful whether a sow will ever exceed her hereditary possibilities in number per litter, but there are many forces that may cause her to fall short of that number. Lack of proper nutrition, failure to have all ova released or fertilized, loss of ova, atrophy of fertilized ova or embryos, and disease may all operate against the complete realization of the hereditary make-up. The age at which a sow farrows, the number of litters she has per year, and certain other environmental conditions may also reduce the litter size. It is interesting to observe that this source of error operates in a compensating direction to that of record falsification, when such exists, and in the end the two may counterbalance, although these physiological and pathological factors operate more often than does the misrepresentation of litter numbers.

After admitting all of these sources of error, but hoping that enough records are made under natural conditions to give the figures an investigational value, there still remains the big question of the geneticist, Does the somatic expression of the character indicate the germinal (zygotic) condition of the individual? In other words, Does the fact that a pig is farrowed in a litter of eight indicate that it will transmit a tendency to produce litters of eight? The answer very evidently is No, and the greater the degree of outcrossing in the ancestral lines, the less reliable an index of heredity the size of litter is. Yet it is the only single index obtainable in the study of herdbook records; so for the present it will have to be accepted for what it is worth.

ADVANTAGES OF LITTER SIZE INHERITANCE STUDIES

Accepting the figures for litter size as reasonably representative of the hereditary constitution, there are a number of reasons that make them desirable material for inheritance studies. The most important of these is the fact that the male mated to a female probably does not affect the number at a birth. Instead the size of litters a sow produces represents the segregation of the tendencies transmitted to her by her father and mother. Suppose a sow produces a litter of four pigs and is herself from a litter of seven, the seven does not determine in any way the four, but instead the segregation of some tendency transmitted by her sire or dam is represented. The only check available on this tendency in her sire is the size of litter in which he was farrowed, while the same holds for the dam, except that her own breeding performance may give an additional idea.

METHOD OF RECORDING THE DATA

The data on the animals studied were recorded as follows, the figures representing the size of litters in which the individuals were farrowed:

		Grandsire
Animal	Dam	4
4	7	Grandam
		9

The size of litters produced by sows whose sires came from litters of four and whose dams came from litters of seven should give an idea (through the variations recorded) of the hereditary factors involved. It is admissible that all grandams or all grandsires farrowed in the same size of litters may be different in hereditary make-up, but there should be enough individuals alike to make the frequency curves at least suggestive. For convenience, the grandparental generation will be lettered "P," the parental generation "F_1," and the filial generation "F_2," although it is clearly to be understood that this notation does not have the regular Mendelian significance.

DEVIATIONS PER GENERATION

The mean size of 1,770 litters in the P generation was 7.84 ± 0.3494. The standard deviation was 2.18 ± 0.2461. This gives a coefficient of variability of 27.80 for this generation.

The mean size of 885 litters for the F_1 generation was 7.82 ± 0.4897. The corresponding standard deviation was 2.16 ± 0.3462. The coefficient of variability here involved was 27.60, practically the same as that of the grandparental generation.

The mean size of 885 litters in the F_2 generation was 7.91 ± 0.4965, while the deviation was 2.19 ± 0.3511, giving a coefficient of variability of 27.55. (See Table I.)

TABLE I.—*Deviation in size of litters in swine*

Generation.	Number of litters.	Mean.	Standard deviation.	Coefficient of variability.
P	1,770	7.84±0.3494	2.18±0.2461	27.80
F_1	885	7.82± .4897	2.16± .3462	27.60
F_2	885	7.91± .4965	2.19± .3511	27.55

The mean litter size is quite constant from generation to generation, and furthermore quite close to that obtained by Surface (26) for the breed in general. If anything of Mendelism is involved here, it is not revealed by this method of treatment, for the standard deviation is so nearly the same for each of the generations involved as to give no hint of segregation. In fact, the coefficients of variability would indicate a slowly increasing degree of homozygosis.

Two interpretations may be placed on these figures. The animals studied are either practically constant from a zygotic standpoint, and the variations in litter size are due to environmental treatment, or else there is so much heterozygosis present in the grandparents that the parents are as much F_2 as F_1 in hereditary make-up. For the present the writers are going to use the latter interpretation, as there is no evidence at hand to support a belief in the former.

TABLE II.—*Deviation in litter size of the offspring from the parental generation in swine*

BOAR 1

Size of litter of parents.		Number of matings.	F_1 generation.		F_2 generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
1	9	1	5	0	9	0
1	4	1	2	0	9	0

BOAR 2

2	5	1	4	0	6	0
2	6	2	8 ±1.43	3 ±1.0117	7.5 ±0.239	.5 ±0.1686
2	8	2	6.5 ± .717	1.5 ± .1737	4 ± .479	1 ± .3372
2	9	1	6	0	6	0

BOAR 3

3	4	3	7 ±0.171	1.41±0.3433	9 ±1.704	4.32±1.194
3	6	2	6.5 ± .239	.5 ± .1686	8 ± .95	2 ± .674
3	7	4	7.25±1.6218	4.8 ±1.1502	7.75± .8869	2.63± .627
3	11	1	6	0	8	0
3	10	2	10 ± .95	2 ± .674	11	0
3	14	1	11	0	6	0

TABLE II.—*Deviation in litter size of the offspring from the parental generation in swine*—Continued

BOAR 4

Size of litter of parents.		Number of matings.	F_1 generation.		F_2 generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
4	3	1	8	0	5	0
4	4	3	7.66±1.032	2.62±0.723	8 ±0.631	1.63±0.442
4	5	5	7.2 ± .9951	3.29± .7022	7.6 ± .5202	1.72± .3671
4	6	7	8 ± .2887	1.13± .2037	7.14± .2836	1.11± .2001
4	7	8	8.5 ± .6863	2.42± .408	5.87± .2726	1.14± .1922
4	8	6	7.83± .5390	1.95± .3801	6.83± .7712	2.79± .5438
4	9	6	7.16± .8127	2.94± .5731	7.33± .4008	1.45± .2826
4	10	4	7.25± .5092	1.51± .3618	6.75± .3844	1.14± .2012
4	12	1	7	0	12	0
BOAR 5						
5	3	1	6	0	12	0
5	4	4	5.75±0.6407	1.92±0.4543	6.5 ±0.3743	1.11±0.2654
5	5	3	7 ± .3195	.81± .2239	6.66± .6666	1.69± .4671
5	6	14	8 ± .3228	1.79± .2282	7.21± .1531	.85± .1083
5	7	12	7.91± .4464	2.29± .358	7.66± .3099	1.59± .2193
5	8	16	7.37± .3979	2.36± .2817	7.56± .408	2.42± .2889
5	9	9	8.11± .3844	1.71± .272	8.22± .5866	2.61± .415
5	10	6	9.33± .5943	2.15± .4191	8.66± .3759	1.36± .2651
5	11	3	6.66± .4891	1.24± .3427	8.33± .3707	.94± .2598
5	12	2	4	0	9	0
BOAR 6						
6	2	1	7	0	8	0
6	3	3	7.66±0.8283	2.1 ±0.5805	3.33±0.6243	1.58±0.4367
6	4	3	4.66±1.1161	2.83± .7822	9.33± .4391	1.24± .3426
6	5	6	8.6 ± .6041	2 ± .4267	6.83± .4533	1.64± .3197
6	6	11	6.72± .4604	2.26± .3251	8.9 ± .4625	2.27± .3263
6	7	18	7.05± .2672	1.68± .1888	8.5 ± .252	1.86± .178
6	8	24	7.58± .3255	2.36± .2300	7.46± .1862	1.35± .1315
6	9	15	8.4 ± .0046	2.74± .0311	8 ± .047	2.7 ± .0314
6	10	8	6.62± .4657	1.71± .2885	7.87± .6075	2.54± .4283
6	11	5	9 ± .9151	2.32± .6414	5 ± .6922	2.29± .4887
6	12	3	7.66± .1853	.47± .1299	8.33±1.136	2.88± .796
BOAR 7						
7	3	1	6	0	10	0
7	4	4	7.5 ±0.8431	2.5 ±0.5802	8.5 ±0.1631	5 ±0.119
7	5	7	7.71± .4202	1.62± .292	7.85± .6106	2.39± .431
7	6	17	7.25± .2177	1.33± .1541	7.58± .239	1.46± .1691
7	7	19	7.52± .3023	1.95± .2135	7.42± .3178	2.05± .2244
7	8	26	8.03± .3637	1.99± .1843	7.8 ± .2545	1.92± .1778
7	9	41	9.29± .1958	1.83± .1363	8.82± .2077	1.94± .1445
7	10	8	8.37± .5333	2.23± .3760	6.62± .4616	1.93± .3254
7	11	16	8.5 ± .2596	1.54± .1838	8.43± .4502	2.76± .3187
7	12	6	8.66± .5943	2.15± .4191	6.5 ± .6109	2.21± .4302
7	13	1	10	0	4	0

TABLE II.—*Deviation in litter size of the offspring from the parental generation in swine*—Continued

BOAR 8

Size of litter of parents.		Number of matings.	F_1 generation.		F_2 generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
8	3	7	7.28±0.1124	0.44±0.0793	7 ±0.3193	1.25±0.2254
8	4	8	8.75± .6721	2.81± .4738	7.75± .5572	2.33± .3928
8	5	11	7.33± .2634	1.91± .1861	8.27± .6377	3.13± .4501
8	6	24	6.91± .2965	2.15± .2094	7.66± .3062	2.22± .2161
8	7	29	7.86± .2394	1.91± .1692	7.82± .277	2.21± .1958
8	8	43	6.58± .2519	2.45± .1783	7.23± .2076	2.02± .1469
8	9	34	8.14± .292	2.52± .2124	8.17± .2294	1.98± .1669
8	10	10	6.7 ± .2219	1.04± .1565	8.4 ± .1462	5.37± .8083
8	11	10	7.8 ±1.024	4.8 ± .7225	8.6 ± .512	2.4 ± .3612
8	12	3	9 ±1.4061	3.55± .9895	9.66± .8415	2.04± .5639
8	13	1	9	0	10	0
BOAR 9						
9	2	1	5	0	9	0
9	3	1	5	0	7	0
9	4	6	7.83±0.4616	1.67±0.3254	7.66±0.4616	2.05±0.3996
9	5	12	7.5 ± .3898	2 ± .2758	6.91± .4482	2.3 ± .3172
9	6	7	6.71± .4444	1.74± .3138	7.14± .6065	2.35± .4238
9	7	32	7.59± .2232	1.87± .1576	8.53± .2495	2.09± .1762
9	8	26	7 ± .2663	2.01± .1861	7.5 ± .3074	2.32± .2148
9	9	35	7 ± .3172	2.95± .2377	8.71± .2158	2.10± .1693
9	10	14	8.66± .4060	2.33± .2874	7.53± .3468	1.99± .2453
9	11	8	7.75± .409	1.71± .2883	8.5 ± .526	2.2 ± .3709
9	12	4	8 ± .1686	.5 ± .119	9.25± .3709	1.1 ± .263
9	13	1	7	0	8	0
9	15	1	6	0	8	0
BOAR 10						
10	1	1	8	0	14	0
10	3	2	9 ±0.4784	1 ±0.3372	7.5 ±1.674	3.5 ±1.1803
10	4	2	9	0	7.5 ±1.674	3.5 ±1.1803
10	5	4	7 ± .4755	1.41± .3372	7.25± .4957	1.47± .3516
10	6	6	7.66± .34	1.23± .2397	8 ± .3897	1.41± .2749
10	7	16	7.93± .3591	2.13± .2542	8.18± .4991	2.96± .3533
10	8	24	8.29± .2978	2.16± .2105	8.26± .3787	2.69± .2676
10	9	11	8.9 ± .6153	3.02± .4343	8.63± .3586	1.76± .2531
10	10	14	6.78± .4128	2.29± .2919	7.57± .2777	1.54± .1963
10	11	4	8.25± .435	1.29± .3085	9.5 ± .5598	1.66± .397
10	12	6	8.5 ± .525	1.9 ± .370	8.63± .5888	2.13± .4151
10	14	1	10	0	8	0
BOAR 11						
11	3	1	9	0	10	0
11	4	1	6	0	9	0
11	5	5	6.8 ±0.0574	1.9 ±0.0405	8.2 ±0.5111	1.69 ±0.3607
11	6	6	8 ± .5749	2.08± .4054	6.33± .4644	1.68 ± .3275
11	7	12	7.33± .3004	1.54± .2124	7.85± .3804	2.007± .2705
11	8	4	8.5 ± .1686	.5 ± .1190	8.75±1.2107	3.59 ± .8586
11	9	6	8.83± .6053	2.19± .4269	8.16± .4809	1.74 ± .3391
11	10	6	7.5 ± .3434	1.25± .2422	8.66± .4646	1.69 ± .3277
11	13	1	6	0	12	0
11	15	1	9	0	9	0

TABLE II.—*Deviation in litter size of the offspring from the parental generation in swine*—Continued

BOAR 12

Size of litter of parents.		Number of matings.	F_1 generation.		F_2 generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
12	2	1	9	0	7	0
12	4	2	9.5 ± 0.238	.5 ±0.118	8.5 ± 0.238	.5 ±0.118
12	5	2	4 ± .956	2 ± .6745	7	0
12	6	1	8	0	10	0
12	7	5	7.6 ± .554	1.85± .3001	9.2 ± .399	1.32± .282
12	8	6	8.82± .384	1.39± .27	8.5 ± .671	2.4 ± .473
12	9	6	6.5 ± .591	2.14± .414	7.83± .387	1.4 ± .272
12	10	1	11	0	10	0
12	11	2	11	0	8.5 ± .717	1.5 ± .505
12	12	1	10	0	12	0
12	13	2	7	0	9.5 ± .717	1.5 ± .505
BOAR 13						
13	6	3	8.66± 0.493	1.25±0.345	8.33± 0.185	0.47±0.129
13	8	2	9.5 ± .717	1.5 ± .505	7 ± .956	2 ± .6745
13	9	2	11.5 ± .717	1.5 ± .505	9 ± .956	2 ± .6745
13	11	1	10	0	6	0
13	12	2	7.5 ± .168	.5 ± .245	10 ± .479	1 ± .337
13	13	5	9.6 ± .526	1.74± .371	10 ± .956	2 ± .6745
BOAR 14						
14	8	2	9	0	10.5 ± 0.239	0.25±0.1686
14	9	1	9	0	11	0
14	12	1	7	0	3	0
BOAR 15						
15	8	1	12	0	8	0

INDIVIDUAL EVIDENCES OF SEGREGATION

Table II is produced by treating the litter size as a detailed character and comparing the parental generation with offspring. The average of the F_1 deviations is 1.87 ± 0.0549, while the F_2 mean deviation is 1.92 ± 0.0582. The probable errors make these two constants overlap, so that the individual treatment when lumped seems no more significant than when the deviations per generation are considered. Yet many individual evidences of segregation exist, and many times the F_2 generation from a particular cross is so small in numbers that only a fragmentary view of the segregable possibilities is obtained.

While it is possible that 90 per cent of the litter sizes in these tables do not represent the exact genetic constitution, yet it is probable that in general the greater the disparity in litter sizes between the two animals in the P generation, the greater will be the expected deviations in the F_2, and the smaller the deviations in the F_1 generation. The following results, Table III, are produced by tabulating the averages of the deviations on this basis.

TABLE III.—*Average deviations in litter size in the F_1 and F_2 generations of swine*

Difference in number of pigs in the two P litters.	0	1	2	3	4	5	6	7	8
F_1 deviations	2.13	1.89	1.93	2.04	2.19	1.82	1.32	1.12	0.5
F_2 deviations	1.91	1.84	2.16	2.10	2.16	1.71	1.72	1.98	.5

A calculation of the probable errors involved in this table shows that only the difference between the F_1 and F_2 deviations where the disparity in litter size is seven pigs is large enough to be mathematically significant. The difference when the parents vary from each other by two pigs and by six pigs is on the border line between significance and nonsignificance, but the five other columns are distinctly unenlightening. Yet, if the difference of two pigs is barred, the results are what might be expected.

One criticism against the preceding method of treatment is thoroughly valid. If swine fertility depends on only one or two genetic factors, it is obvious that the point at which the difference between the two parents occurs is more important than the degree of difference. For example, if there is a physiological division point between two hereditary factors at six pigs, then a difference of two or even of four below six pigs might not be significant, while a difference of one more or one less in a litter of six or seven pigs would be thoroughly significant. An examination of the data from this point of view is now in progress, but it is probable that the key to the situation will only be discovered by breeding experiments.

CURVES OF LITTER FREQUENCIES

The distribution of the different sizes of litters in the three generations is given in Table IV.

TABLE IV.—*Litter frequencies in swine*

Generation.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P Expected	0.11	1.5	9.8	39	108	216	324	370	324	216	108	39	9.8	1.5	0.11
P Actual	3	9	30	80	124	198	300	362	318	162	91	59	26	6	3
F_1 Expected	.05	.75	4.9	19	54	108	162	185	162	108	54	19	4.9	.75	.05
F_1 Actual	0	5	14	32	69	122	149	161	149	85	62	23	8	4	2
F_2 Expected	.05	.75	4.9	19	54	108	162	185	162	108	54	19	4.9	.75	.05
F_2 Actual	0	4	17	32	63	107	154	172	135	95	59	30	11	3	3

Figures 1, 2, 3, and 4 show the curves for the litter frequencies in the three generations and indicate how close the actual numbers of litters come to the binomial curve $(x+y)^{14}$. It is perhaps incorrect to call the theoretical frequencies recorded in Table IV "expectations," unless it is clearly understood that they are the expectations founded on the nearest binomial. There is nothing in the inheritance to make them true expectations from an experimental standpoint.

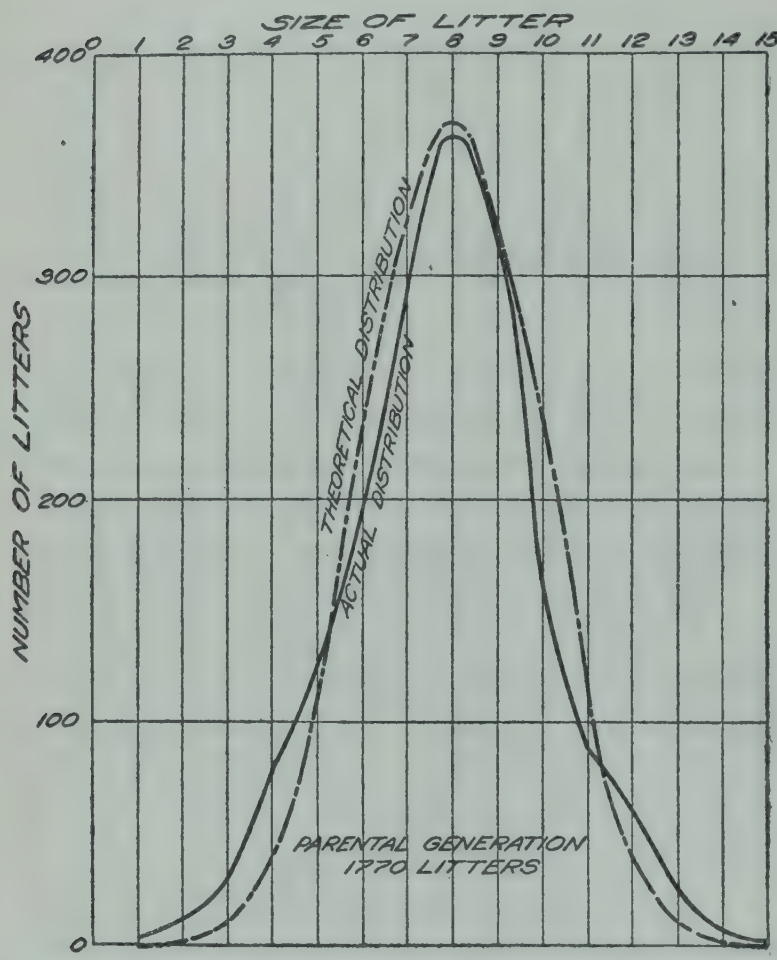


FIG. 1.—Curve of litter frequencies in the P generation of swine.

The curve of the actual distribution spreads out at the extremities much wider than should be expected on a chance basis. This is true in regard to both extremes of the curve and makes it appear as though the curve were compound—i. e., the sum of several curves having separate means from which deviations take place. An analysis on this basis gives two small curves at the extremes, which, while they do not give perfect Gaussian distributions, are characteristic enough to make the assumption of another mean for each perfectly valid. The modes of these three curves are as follows:

Curve 1	4 pigs per litter.
Curve 2	8 pigs per litter.
Curve 3	12 pigs per litter.

It is premature to announce that these modes represent centers of deviation for genetic factors, although a casual observation of the individual data makes it seem that this condition may exist. Furthermore, the mode of curve 1 corresponds to the degree of fertility which Simpson states is characteristic of the wild hog, while the mode of curve 3 is very close to that of the Tamworth, the most fecund of domestic breeds. This indicates that the two may represent basic and improved factors for fertility, respectively, while curve 2 represents heterozygous conditions.

Before these curves can be accepted as more than merely suggestive a further analysis must be made. There is a significant deviation from expectancy in the right-hand branch of the curve of the total population, which persists even after the separation into three curves. In figure 4 this deficiency is located in the left-hand branch of curve 3, but the minus deviations may just as logically belong in the right-hand branch of curve 2, suggesting that it also may be compounded of two curves dependent on a genetic factor not disclosed thus far.

Paralleling this study some actual matings of swine have been planned and are in progress.

SUMMARY

(1) Fertility in swine offers favorable material for the study of quantitative inheritance, because the units of deviation are discrete.

(2) Biometric studies of litter size with mother and daughter have indicated a small degree of inheritance.

(3) Crosses of breeds having different mean

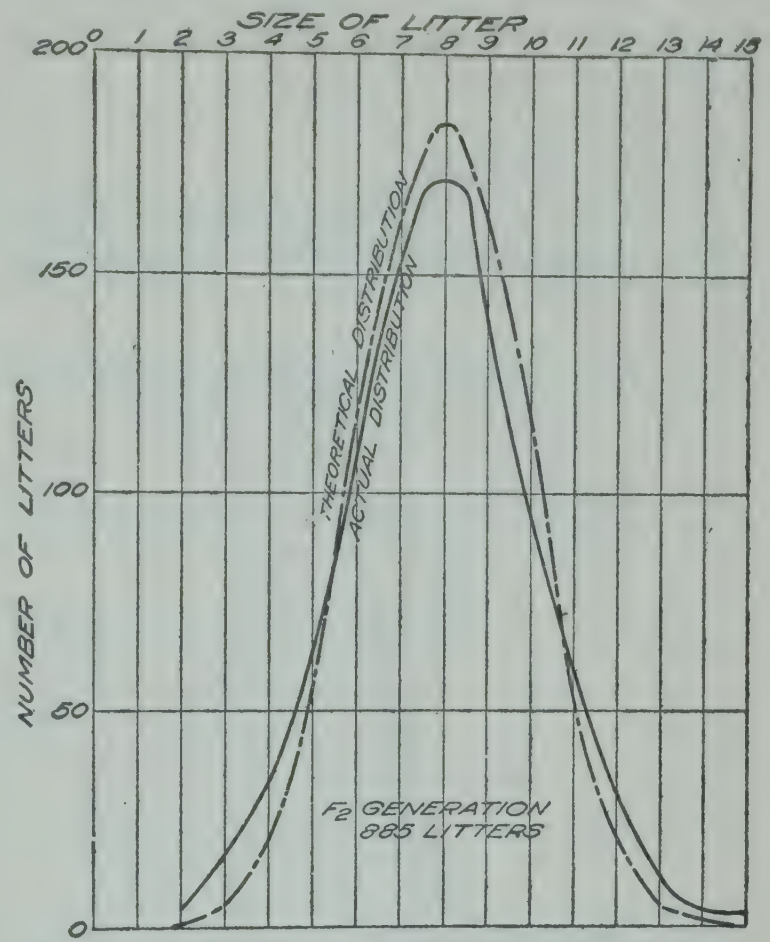


FIG. 2.—Curve of litter frequencies in the F_1g eneration of swine.

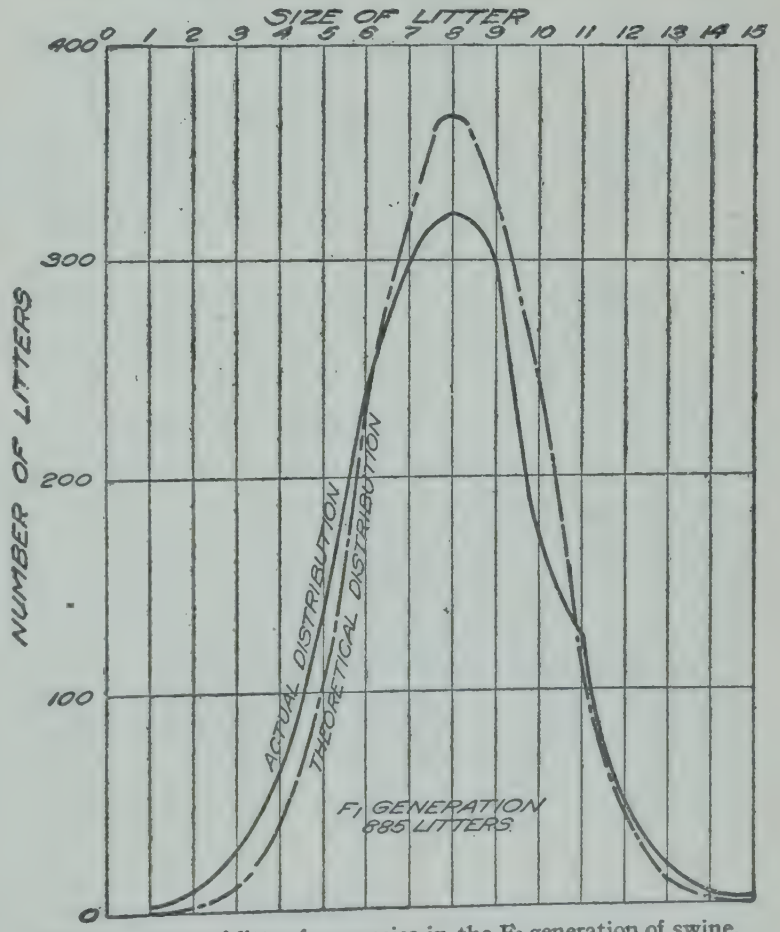
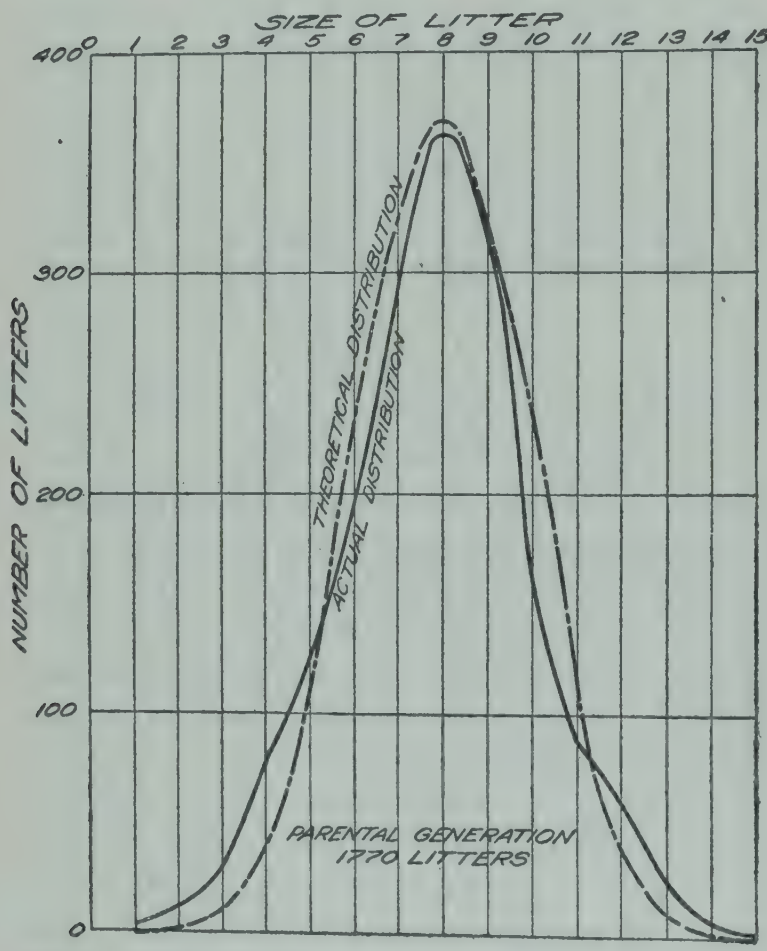


FIG. 3.—Curve of litter frequencies in the F_2 generation of swine.

Figures 1, 2, 3, and 4 show the curves for the litter frequencies in the three generations and indicate how close the actual numbers of litters come to the binomial curve $(x+y)^{14}$. It is perhaps incorrect to call the theoretical frequencies recorded in Table IV "expectations," unless it is clearly understood that they are the expectations founded on the nearest binomial. There is nothing in the inheritance to make them true expectations from an experimental standpoint.



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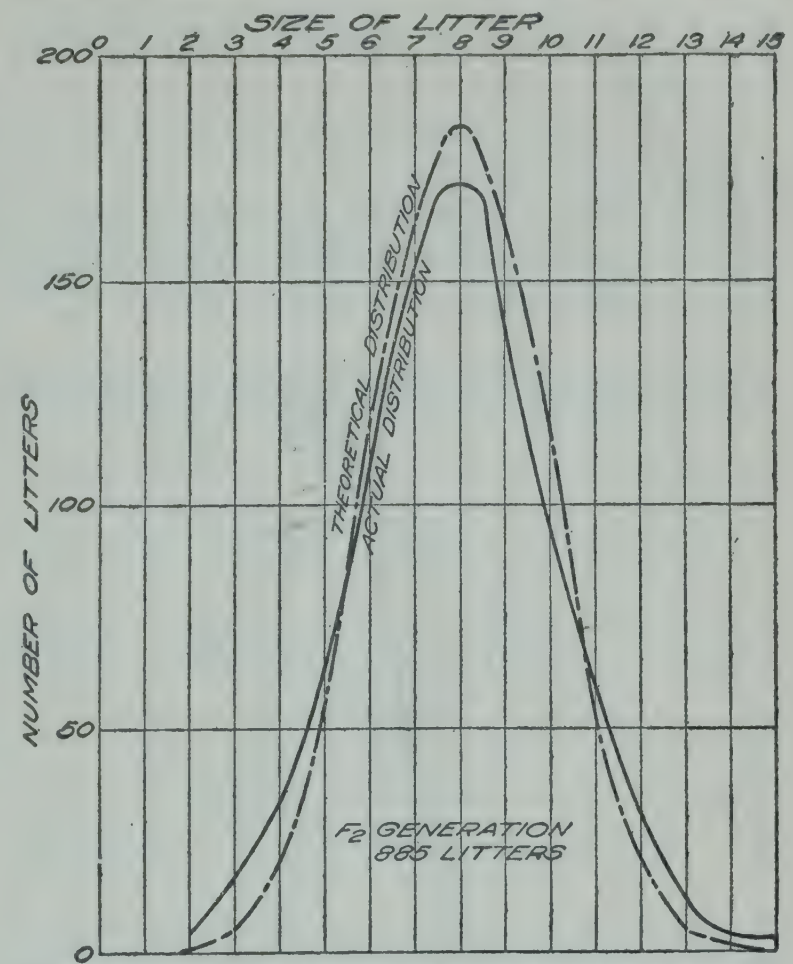

FIG. 2.—Curve of litter frequencies in the F_1g eneration of swine.

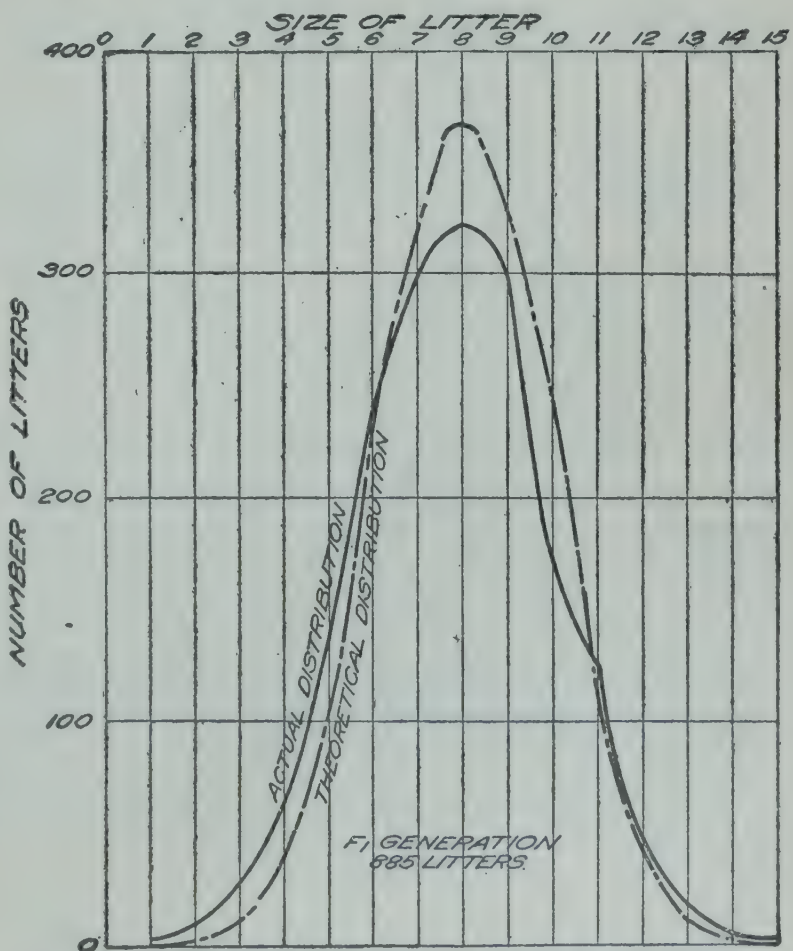

FIG. 3.—Curve of litter frequencies in the F_2 generation of swine.

SUMMARY

(1) Fertility in swine offers favorable material for the study of quantitative inheritance, because the units of deviation are discrete.

(2) Biometric studies of litter size with mother and daughter have indicated a small degree of inheritance.

(3) Crosses of breeds having different mean

litter sizes have suggested that segregations of fecundity factors may take place.

(4) Numerous nongenetic factors limit the full expression of the inborn possibilities of fertility.

(5) Certain few somatic characters may be correlated either in a physiological or genetic manner with the different degrees of fecundity, but the bulk of characters usually assumed to be so related are probably entirely independent of it.

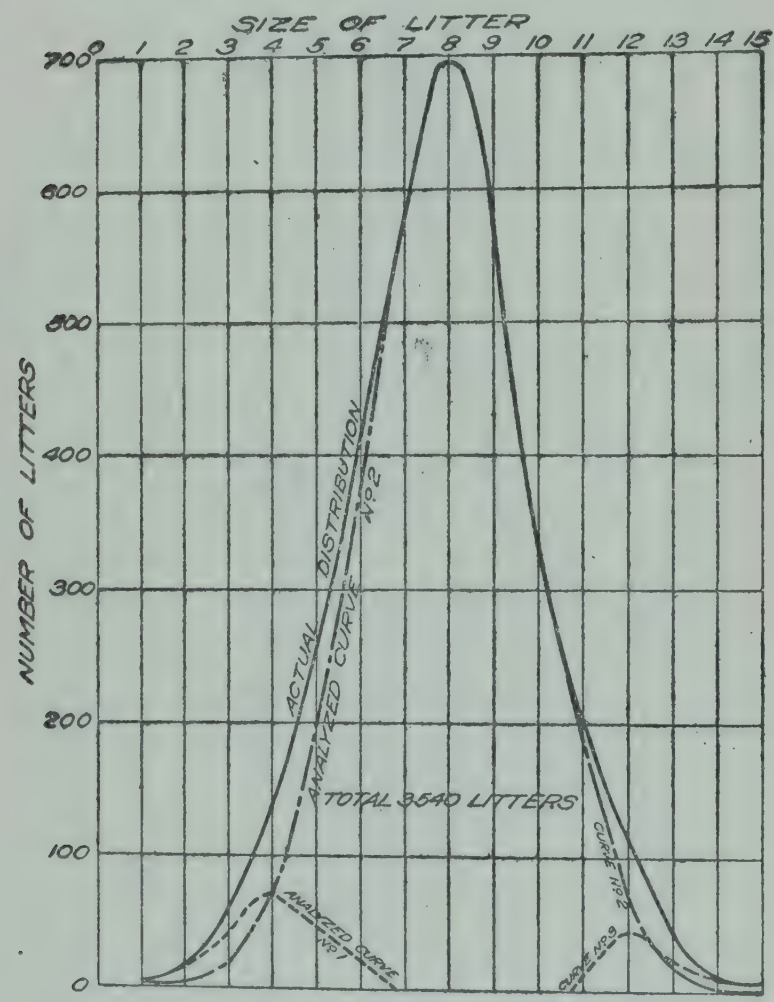

FIG. 4.—Diagram of the combined litter frequencies for the three generations of swine analyzed into its component curves.

(6) Herdbook data on the fertility of swine present sources of error, but the percentage of error is low enough to permit the statistics to be suggestive.

(7) Numerous influences exist which lower the size of litter, which sources of error may operate in a manner compensatory to those just mentioned.

(8) It is questionable whether the size of litter represents the hereditary factors transmitted, but the somatic character was perforce accepted at face value in these studies.

(9) There is no reduction in variability in the litter sizes of the dams as compared with the grandparents or progeny, as would result if there were homozygous differences for fertility in the grandparents. Hence, the fertility deviations are either nongerminal or else the degree of heterozygosis is so great in the grandparents that no increased variability in the F_2 generation is possible. The latter explanation is probably the correct one.

(10) The frequency curves for the 3,540 litters studied make it appear that there are at least three centers of deviation in swine fertility. These centers possibly correspond to genetic factors involved in the inheritance of fecundity.

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RELATION OF GREEN MANURES TO THE FAILURE OF CERTAIN SEEDLINGS

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INTRODUCTION

In a previous report it has been shown that if green manures are turned under and the soil planted immediately, a decrease in germination may result. For example, a 20-acre field, half in crimson clover (*Trifolium incarnatum*) and half in fallow, was plowed and planted to cotton (*Gossypium* spp.) (17, p. 26).[2] On the crimson clover plot the cotton failed almost completely to germinate. Here and there a few crippled seedlings appeared, while on the fallowed plot normal germination occurred. Seed from the same lot was used on both plots. The green manure in some way seriously affected the germination of the cottonseed. Three weeks later the green-manure plot was again seeded to cotton. Germination at this time was perfectly normal. Apparently the harmful factor disappeared during the interval of three weeks.

A more extensive study of the substances affecting seed germination and of the factors involved was deemed advisable. The controlling idea in this investigation was a study of the effect of green manures on the germination of different seeds. In determining the percentage of germination, only those seedlings that appeared above the surface are recorded.

The amount of green manure used was determined from the following calculation: A good crop of clover should yield from 4 to 5 tons of undried green hay per acre. If 1 acre of soil 3 inches deep weighs 1,000,000 pounds, then 1 per cent of green clover is comparable to the amount employed under field conditions. Except in rare cases this amount of green manure was used in all of the laboratory studies. The green plant tissue was cut just before blooms began to form, finely chopped, and mixed thoroughly with Miami silt loam soil from the Experiment Station farm. The soil moisture was maintained at 50 per cent saturation. All tests of germination are recorded in percentages. Photographs were made of the young seedlings two weeks after planting.

EFFECT OF GREEN MANURES ON THE GERMINATION OF VARIOUS SEEDS

Since it has been shown that seeds of different plants vary widely in chemical composition, it is very probable that they will react differently toward green manures. This experiment was planned to test the effect

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] Reference is made by number to "Literature cited," p. 1175–1176.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. V, No. 25
Mar. 20, 1916
Wis.—4

of decomposing plant tissue on the germination of buckwheat, castor beans, corn, crimson clover, flax, hemp, lupines, mustard, oats, peanuts, soybeans, sunflower, and wheat. The percentage composition of these seeds is given in Table I.

TABLE I.—*The percentage composition of various seeds (11, 20)*

Name.	Fat.	Crude protein.	Nitrogen-free extract.	Crude fiber.	Ash.
Castor bean (*Ricinus communis*)	51.37	18.75	1.5	18.1	3.1
Peanut (*Arachis hypogaea*)	45	25	18	.....	2 to 5
Flax (*Linum usitatissimum*)	33.7	22.6	23.2	7.1	4.3
Hemp (*Cannabis sativa*)	32.58	18.23	21.06	14.97	4.24
White mustard (*Brassica alba*)	29.66	27.59	20.83	10.27	4.47
Sunflower (*Helianthus annuus*)	28.79	16.3	17.28	27.9	3.3
Cotton (*Gossypium herbaceum*)	20.86	19.69	23.43	21.1	3.8
Soybean (*Glycine soja*)	17.00	35.00	26.00	5 to 6	4.5
White lupine (*Lupinus albus*)	6.79	28.78	33.65	11.92	2.99
Oat (*Avena sativa*)	5.27	10.25	59.68	9.97	3.02
Corn (*Zea mays*)	4.5	9.5	68.5	2.18	1.6
Buckwheat (*Fagopyrum tataricum*)	2.68	11.41	58.79	11.44	2.38
Wheat (*Triticum sativum*)	1.65	10.93	70.01	2.12	1.92

The seeds are grouped according to fat content; those richest in fat are given first. The marked difference in the chemical composition of various seeds is very noticeable. For instance, castor beans contain more than 50 per cent of fat, while oats contain less than 2 per cent.

According to Nobbe (16, p. 173), seeds rich in oil require more oxygen for germination than starch seeds. In Tables II, III, and IV data are presented concerning the effect of green manures on various seeds. In every case the seeds were tested under identical conditions. The figures of Table II show the effect of 1 per cent of green clover on the germination of buckwheat, corn, hemp, lupine, and sunflower.

TABLE II.—*Effect of green clover on the germination of various seeds*

No.	Seed.	Treatment.	Germination.		
			1 week.	2 weeks.	Relative.
			Per cent.	*Per cent.*	*Per cent.*
1	Buckwheat	None	75	90	100
2	.....do	1 per cent clover	90	90	100
3	Corn	None	100	100	100
4	.....do	1 per cent clover	95	100	100
5	Hemp	None	95	95	100
6	.....do	1 per cent clover	65	65	68
7	Lupine	None	75	80	100
8	.....do	1 per cent clover	60	60	75
9	Mustard	None	95	95	100
10	.....do	1 per cent clover	55	55	58
11	Sunflower	None	90	90	100
12	.....do	1 per cent clover	90	90	100

The average percentage of germination in duplicate pots, after one and two weeks, is recorded in Table II. The last column gives the relation between the treated and untreated seeds. A glance at the figures shows clearly that buckwheat, corn, and sunflower were not injured by green manures. On the other hand, hemp and mustard were seriously injured; the latter showed the greatest loss. Lupines are not so sensitive as mustard or hemp toward green manure, although a slight decrease in germination is noted.

As regards fat content, it will be seen that with the exception of sunflower those seeds rich in oil are the most sensitive to green manuring. The very quick germination of sunflower seed may explain their resistance to the injurious factor.

Table III presents data to show the striking difference in behavior of fat and starch seeds toward green manures. A comparison of the injury resulting from the use of green clover and green oats is made.

TABLE III.—*Effect of green clover and oats on the germination of cottonseed and wheat*

No.	Seed.	Treatment.	Germination.			
			1 week.	2 weeks.	3 weeks.	Relative.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	Cotton.....	None.....	85	92. 5	92. 5	100
2	.....do.....	1 per cent of oats.....	45	65	65	70
3	.....do.....	1 per cent of clover..	17. 5	17. 5	17. 5	19
4	Wheat.....	None.....	95	100	100	100
5	.....do.....	1 per cent of oats.....	85	90	90	90
6	.....do.....	1 per cent of clover..	85	85	85	85

The germination of cotton was seriously injured by the presence of green manures; the green clover was much more harmful than oat tissue. Wheat was little affected by the use of green manure. The data confirm the results of the preceding test—that is, that seeds rich in oil are especially sensitive to green manures. It appears that the percentage of injury depends to a certain degree on the source of the plant tissue. Plate LXXXIII, figure 1, is reproduced from a photograph of cotton seedlings two weeks after planting. In order to make the seedlings more visible, a thin layer of white quartz sand was poured upon the surface of the soil.

With soybeans in place of wheat, this experiment was repeated, as shown in Table IV.

TABLE IV.—*Effect of green clover and oats on the germination of cottonseed and soybeans*

No.	Seed.	Treatment.	Germination.			
			1 week.	2 weeks.	3 weeks.	Relative.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	Cotton....	None.....	95	100	100	100
2	.....do.....	1 per cent of oats.....	35	35	35	35
3	.....do.....	1 per cent of clover..	.....	10	10	10
4	Soybean...	None.....	100	100	100	100
5	.....do.....	1 per cent of oats	40	40	40	40
6	.....do.....	1 per cent of clover..	30	60	60	60

Here it was again found that the oil seeds are very sensitive to green-manuring. Soybeans are more resistant to this injury than cotton.

As regards the source of the green manure, the results of numerous tests indicate that clover causes a greater loss than oat tissue. An exception to this is found with soybeans (Table IV). No satisfactory explanation has been found for the different action of these two substances. The average of three total-nitrogen analyses shows that clover contains 80.27 per cent of moisture and 4.8 per cent of protein (dry basis). The oats contained 82 per cent of moisture and 3.96 per cent of protein. Chemical analyses fail to disclose any very striking differences between the clover and oat tissue. Indeed, the protein content is nearly the same in both substances. It is possible that the nitrogen of legumes is more available than that of nonlegumes (14). It was noticed repeatedly that clover tissue decomposes more rapidly than oat tissue.

EFFECT OF TIME OF PLANTING AND QUANTITY OF GREEN MANURE ON THE GERMINATION OF COTTON SEED

Ten half-gallon jars were filled with soil and treated as shown in Table V.

TABLE V.—*Effect of time of planting and quantity of clover on the germination of cottonseed*

No.	Treatment.	Germination.							
		Planted immediately.				Planted two weeks later.			
		1 week.	2 weeks.	3 weeks.	Relative.	1 week.	2 weeks.	3 weeks.	Relative.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	None.....	90	90	90	100	90	95	95	100
2	0.25 per cent of clover..	60	60	60	66	90	95	95	100
3	0.5 per cent of clover...	50	50	50	55	80	95	95	100
4	1.0 per cent of clover...	35	35	35	38	100	100	100	100
5	2.0 per cent of clover...	.....	.....	.....	.....	75	85	85	89
6	3.0 per cent of clover...	.....	.....	.....	.....	70	85	85	89

From the data of this experiment it is very evident that the serious injury caused by green manures is only temporary. Two weeks after the green manure was turned under, the conditions that affect seed germination disappeared. Aside from the temporary nature of the injurious agent, it will be seen that the percentage of injury is fairly proportionate to the amount of green clover used. In the presence of 0.25 per cent, the rate of germination was decreased 34 per cent, while more than 1 per cent of green manure entirely prevented germination. A comparison of the effect of green manures in different stages of decomposition on cotton germination is shown in Plate LXXXIII, figures 2 and 3.

FIELD EXPERIMENTS WITH GREEN MANURES

Early in the spring of 1914 a series of plot experiments with various seeds was made. For this purpose a good clover sod from the Experiment Station farm, near Madison, Wis., was chosen. This sod was divided into three equal sections: *A*, Clover; *B*, oats; and *C*, unplanted. The sections were subdivided into six plots, as shown in Table VI. Section *A* was allowed to remain in clover, while *B* and *C* were plowed, section *B* planted to oats, and *C* left without any crop. When the oats in section *B* and the clover in section *A* were partly in bloom, the soil was plowed and prepared for planting. One half of each section was planted immediately, the other half 25 days later. It was arranged to study the effect of clover and oat tissue on the germination of cotton, corn, hemp, oats, and soybeans. The same weight of seed was planted in each plot. The results of this series of tests are given in Tables VI and VII.

TABLE VI.—*Effect of green clover on the germination of various seeds*

No.	Seed.	Planted immediately after turning under.				Germination of seed planted 25 days after turning under.	
		With clover.		Unplanted.		With clover.	Unplanted.
		Seed germination.	Weight.	Seed germination.	Weight.		
			Pounds.		*Pounds.*		
1	Cotton	60	.....	91	.....	190	210
2	.....do	71	.....	129	.....	202	218
3	Corn	76	21	79	27	68	75
4	Hemp	Few.	8	Many.	27	1,050	1,130
5	Oats	505	.....	474	.....	Fine.	Fine.
6	Soybean	58	4	83	5.5	83	88

TABLE VII.—*Effect of oats on the germination of various seeds*

No.	Seed.	Germination of seed.			
		Planted immediately after turning under.		Planted 25 days after turning under.	
		With oats.	Unplanted.	With oats.	Unplanted.
1	Cotton	100	210	134	140
2	.....do	117	218	125	131
3	Corn	62	75	72	73
4	Hemp	450	1,130	210	320
5	Oats	Many.	Many.	Many.	Many.
6	Soybean	35	88	39	40

From these tables it will be seen that green manures seriously injure the germination of cotton, soybeans, and hemp, while corn and oats are not affected. The diminished germination is not confined to clover tissue, but is noted with oats. This effect of the plant tissue on germinating seeds is also observed in the weight of harvest. Unfortunately, because of climatic conditions, the cotton could not be grown to maturity. On adjoining plots, where the green manure was allowed to decompose for 25 days before planting, no injury was observed.

The field data show (1) that green manures largely prevent the germination of certain oil seeds, and (2) that the unfavorable condition is only temporary.

NATURE OF THE INJURIOUS AGENT

There are a number of possible causes that might account for the destructive influence of green manures on seed germination:

First, the green manure greatly increases the number and variety of micro-organisms. The organisms on the plant tissue may be harmful, or conditions proper for the development of harmful organisms may arise.

Second, the large gain in number of organisms, after the addition of green manure, results in a possible accumulation of substances toxic to germination—for example, poisonous by-products of decomposition, as alkali or acid.

Third, the rapid multiplication of micro-organisms, which results in an increased metabolism, causes soil oxygen to be consumed and carbon dioxid to be given off. Such loss in oxygen and gain in carbon dioxid might conceivably retard or prevent germination. If it is assumed that oil seeds require more oxygen for germination than starch seeds, the third supposition should apply particularly to seeds rich in fat (16, p. 173).

EFFECT OF SOIL TYPE

In order to ascertain the relation to soil type of the agent causing a decrease in germination, a series of tests was made. Four soil types were used: Colby silt loam, Miami silt loam, Sparta acid sand, and neutral sand. The results of the first test are given in Table VIII.

TABLE VIII.—*Effect of green manure on the germination of cottonseed*

No.	Soil.	Treatment.	Germination.			Relative.
			1 week.	2 weeks.	3 weeks.	
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*
1	Colby silt loam (acid).....	None.....	90	90	90	100
2	.....do.....	1 per cent of clover.	35	45	50	55
3	Miami silt loam.....	None.....	75	75	75	100
4	.....do.....	1 per cent of clover.	35	35	35	50
5	Miami silt loam, half sand....	None.....	95	95	95	100
6	.....do.....	1 per cent of clover.	45	45	45	50
7	Sand.....	None.....	80	80	80	100
8	.....do.....	1 per cent of clover.	90	90	90	112
9	Sparta acid sand.....	None.....	80	80	85	100
10	.....do.....	1 per cent of clover.	70	70	70	82

For the purpose of securing variation in texture, dilutions with Miami soil and quartz sand were made. From the data obtained, it seems that the property of reducing seed germination is common to both silt loams, but is absent or almost inactive in the sands. Since the relative decrease in germination is approximately the same with Miami or Colby silt loam, it appears that soil reaction is not one of the controlling factors. In neutral or acid sand no decided injury was noted. The results of a second series of tests confirm the above statement. Just why sandy soil should prove less efficient than the loams is not evident from the data, unless it is due to the absence of the injurious factor.

EFFECT OF POSITION OF GREEN MANURE

It was arranged to study the effect on seed germination of plant tissue at different depths. Green clover was added at the rate of 1 per cent. The results secured were as follows: When the green manure was placed in the bottom of the jar, 80 per cent of cotton germinated; in the middle, none germinated; on top, 10 per cent germinated. It is evident that green clover must be in close contact with the seed in order to be effective. This may be shown by wrapping cotton seeds with clover leaves. One or two clover leaves greatly injured seed germination. Plate LXXXIII, figure 4, shows the effect of position of green manure on seed germination.

EFFECT OF INCREASED AERATION

In view of the different action of green manures in compact and open soils, it was decided to make a series of tests under conditions that tend to remove gaseous substances. For this purpose, specially designed jars with openings in their bottoms were employed. By means of a glass tube connected with the bottoms of the jars, air was forced through the soil. In these tests air was allowed to pass through the soil for 20 to 30 minutes every day. A comparison of germination in the aerated and unaerated soils failed to show any difference. Change in soil air did not lessen the injury.

EFFECT OF TEMPERATURE

It is a well-known fact that slight changes in temperature often greatly increase or decrease the growth of micro-organisms. Accordingly a test was made with three variations in temperature.

TABLE IX.—*Effect of temperature on germination of cottonseed*

No.	Treatment.	Temperature.	Germination.		Relative.
			4 days.	8 days.	
		° C.	*Per cent.*	*Per cent.*	*Per cent.*
1	None	25	85	85	100
2	1 per cent of clover	25	55	55	64
3	None	30	95	95	100
4	1 per cent of clover	30	35	35	36
5	None	37	100	100	100
6	1 per cent of clover	37	80	80	80

About 30° C. seems to give the greatest injury; lower or higher temperatures fail to cause so great a decrease in germination.

EFFECT OF CERTAIN DECOMPOSITION PRODUCTS

In the decomposition of plant tissue many substances are liberated—e. g., ammonia and carbon dioxid. The relation of ammonium hydroxid to seed germination has been studied by Bokorny (3; 4, p. 37). He found that small quantities of ammonium hydroxid, 0.02 per cent, greatly retarded the germination of cress. It seems that the active protein of the cell is very sensitive to ammonia.

AMMONIUM HYDROXID

A series of tests was made using from 0.1 to 0.01 per cent of ammonium hydroxid. Four different seeds, cotton, corn, soybeans, and wheat, were allowed to germinate between cloths saturated with the varying concentrations of ammonium hydroxid. It was found that 0.05 or 0.01 per cent proved injurious, while 0.1 per cent prohibited all germination.

Since it was established that ammonia is harmful to seed germination, another test was carried out to study the ammonia produced by micro-organisms. The results of this study are shown in Table X.

TABLE X.—*Effect of sugar and of clover on ammonification*

Time in 2-day intervals.	Ammonia nitrogen in 100 gm. of soil.		
	No treatment.	1 per cent of sugar added.	1 per cent of clover added.
	Mgm.	*Mgm.*	*Mgm.*
1	1. 98	2. 0	3. 3
2	.....	2. 1	4. 3
3	.....	1. 96	2. 8
4	.....	1. 4	2. 4
5	.....	1. 4	2. 5
6	1. 90	2. 5	2. 6
Total	.....	11. 36	17. 9

Since ammonia formation is largely a product of bacterial action, it was thought that sugar or green manure would cause an enormous increase in this substance. The data of Table X show a slight gain in ammonia in the treated soils, but the amount is far too small to affect germination seriously

CARBON DIOXID

It was found that carbon dioxid, when added in large quantities, retards germination but does not cause the seeds to decay. As soon as the carbon dioxid is removed, germination proceeds in a normal manner. In Table XI is given the periodic evolution of carbon dioxid from soil treated with 1 per cent of sugar and 1 per cent of clover.

TABLE XI.—*Effect of sugar and clover on carbon-dioxid evolution*

Time in days.	Carbon dioxid in 100 gm. of soil.		
	No treatment.	1 per cent of sugar added.	1 per cent of clover added.
	Mgm.	*Mgm.*	*Mgm.*
1	4. 62	22. 0	16. 02
2	6. 82	17. 2	12. 7
3	9. 46	36. 52	22. 0
4	7. 21	37. 84	22. 75
5	7. 57	33. 97	22. 7
6	7. 74	29. 35	24. 2
7	7. 65	26. 40	24. 42
8	9. 68	25. 30	22. 22
Total	60. 75	228. 58	167. 01

From the data in this table it is evident that the amount of carbon dioxid evolved in the presence of sugar or clover is far too small to exert a marked effect on germinating seeds.

CALCIUM CARBONATE

It is well known that free acids greatly retard or prohibit germination (3; 4, p. 37). Aside from the direct effect on seeds, an acid reaction may favor the growth of injurious micro-organisms. Accordingly, two series of tests were made, using a neutral and an acid soil with varying amounts of limestone ($CaCO_3$). The results of the first test are given in Table XII.

TABLE XII.—*Effect of green clover and calcium carbonate on the germination of cottonseed*

No.	Treatment.	Germination.			
		1 week.	2 weeks.	3 weeks.	Relative.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	None	85	85	85	100
2	1 per cent of clover	55	55	55	64
3	1 per cent of clover, 0.1 per cent of calcium carbonate	35	.....	.....	40
4	1 per cent of clover, 0.2 per cent of calcium carbonate	15	.....	.....	17
5	1 per cent of clover, 0.5 per cent of calcium carbonate	15	.....	.....	17
6	1 per cent of clover, 1.0 per cent of calcium carbonate	10	.....	.....	11

The data show clearly that limestone in concentrations of from 0.1 to 1 per cent seriously injured the germination of cotton. The seedlings from limed soils died during the first or second week. A second test, similar to the above, was carried out, using acid soil. Here again calcium carbonate seemed to stimulate the injurious factor.

EFFECT OF HEAT

The results of previous tests indicate very strongly the biological nature of the factor injurious to germination. For example, reduced germination is largely associated with the first stages of decomposition. Second, the data seem to exclude the possibility of harmful gaseous products. It is conceivable that in the early stages of decomposition green tissue is favorable to the growth of certain organisms injurious to germination. Accordingly, a series of experiments were made in which the amount and form of green manure applied, the seed, and the biological factors were modified. From 1.5 to 3 per cent of green manure was added. To remove the biological factor, the jars and contents were sterilized in the autoclave at 15 pounds' pressure for two hours. The results of this study were recorded by photographs. Reading from left to right (Pl. LXXXIV, fig. 6), the jars were treated as follows: A, none,

unsterilized; *B*, 1.5 per cent of green manure, sterilized; *C*, 1.5 per cent of green manure, unsterilized; *D*, 3 per cent of green manure, sterilized; *E*, 3 per cent of green manure, unsterilized. The soil shown in the pots in Plate LXXXIII, figure 5, was treated with green oats, in Plate LXXXIV, figure 6, with green clover. Since the corn and wheat did not show any injury, these illustrations were not reproduced. The data from cotton, clover, and flax are presented in Plate LXXXIV, figures 1, 2, 3, 4, and 5. A glance at the seedlings in the sterilized soil shows conclusively that heat removes or renders inactive the harmful factor. The percentage germination of all crops in the sterilized green-manure soil was equal to that of the untreated controls. Apparently, sterilization has in some way prevented any injury from green-manuring. This is true with 1.5 or 3 per cent of green manure. When repeated, the same results were obtained. These data are given in Table XIII. All of the results point to an injurious agent of biological nature.

TABLE XIII.—*Effect of heat on the germination of cottonseed*

Letter.	Treatment.	Germination.			Relative.
		1 week.	2 weeks.	3 weeks.	
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
A	None	95	100	100	100
B	Sterilized	85	85	85	85
C	1 per cent of clover	.....	10	10	10
D	1 per cent of clover sterilized.	80	80	80	80
E	1 per cent of oats	35	35	35	35
F	1 per cent of oats sterilized...	85	90	90	90

SOURCE OF INJURIOUS AGENT

When portions of diseased seedlings are used to inoculate sterilized green-manured soil, the germination of oil seeds is greatly reduced. Numerous tests show that the harmful agent is readily transferred. From the data it must be concluded that the injury to seed germination is biological, probably due to bacteria or fungi. To study the nature of the agent, a series of tests was made with different micro-organisms.

EFFECT OF BACTERIA

In this series of tests bacteria from seed, from green manure, and from soil were studied. From the nature of the seed coat of cotton it is no doubt very rich in a number of bacteria. According to plate counts, the number of micro-organisms on cottonseed is over 122,000 per gram, or an average of nearly 11,000 organisms to one seed. A comparison of the germination of cottonseed free of bacteria and with bacteria, in unsterilized green-manured soil, did not disclose any difference in germination. The bacteria were removed (2) by exposing the seed to the action of hot mercuric chlorid ($HgCl_2$) or concentrated sulphuric acid (H_2SO_4). The use of sulphuric acid offers an easy and satisfactory method of

removing micro-organisms from cottonseed. The seeds were placed in a large glass-stoppered bottle containing concentrated sulphuric acid and glass beads. After shaking for two minutes, the seeds were removed with a platinum loop and washed in boiled water. From the data it seems that infection is from some source other than the seed.

It has been shown repeatedly that the addition of green manure to soil is followed by an enormous increase in the number of bacteria. Aside from the increase in bacterial food, the green manure carries with it a great number of bacteria (6, 8, 21). Tests with bacteria-free green manures failed to eliminate the injury.

About 16 pure cultures of bacteria were isolated from diseased seeds and green-manured soil. In order to test the effect of these various micro-organisms on germination, sterilized green-manured soil was inoculated with the various species of bacteria and seeded. The tests were carried out in triplicate, using bacteria-free seed of cotton, peanut, and soybeans. Here, again, bacteria failed to show any effect on the germination of oil seeds. In addition to the pure cultures used in the above experiment, a study was made with four laboratory stock cultures, *Bacillus fluorescens liquefaciens*, *B. subtilis*, *B. mesentericus vulgatus*, and *Streptothrix bucallis*. Heavy inoculations of these organisms did not injure the germination of cottonseed or soybeans. This agrees with the results of earlier workers (12, 13, 15, 18)—that is, bacteria grown on rich nitrogenous media do not injure seed germination. An exception to this is noted with cracked or injured seeds.

EFFECT OF FUNGI

From a study of tests carried out with various combinations of sterilized soil, green manure, and seeds free of micro-organisms, it was found that the harmful factor occurs chiefly in soil. The data in Table XIV show very conclusively the position of injury.

TABLE XIV.—*Effect of fungi on the germination of cottonseed*

No.	Treatment.	Germination.			
		1 week.	2 weeks.	3 weeks.	Relative.
1	Sterilized soil, 1 per cent of sterilized clover.	*Per cent.* 20	*Per cent.* 70	*Per cent.* 70	*Per cent.* 100
2	Sterilized soil, 1 per cent of unsterilized clover.....	15	45	45	64
3	Unsterilized soil, 1 per cent of sterilized clover.....	.....	.....	.....	.....
4	Unsterilized soil, 1 per cent of unsterilized clover.....	.....	.....	.....	.....

It seems that the harmful agent is found both in soil and in plant tissue, although it is much more prevalent in soil. The results of later tests confirm this statement.

According to many investigators, fungi may injure seed germination (1, p. 30–39; 7, 12, 15). For example, Muth (15) found *Aspergillus niger* harmful to the germination of various seeds, while Atkinson (1, p. 30–39) and Bolley (5, p. 25–27) report a destruction of cotton and flax seedlings by species of Rhizoctonia and Fusarium.

Since it is established that certain soil fungi are injurious to very young seedlings, the question arises as to the occurrence and growth of parasitic fungi in green-manured soil. An experimental study of the occurrence of fungi in green-manured soil was made. Microscopical examinations of the diseased seeds showed the presence of many fungi on the primary root tip. Although no systematic study was made, some of the forms showed certain characteristics of the genus Rhizoctonia and others of the genus Fusarium. From portions of the diseased tissue plates were poured. In this way several species of fungi were isolated. These are described under laboratory numbers. All attempts to secure a pure culture of any species of Rhizoctonia failed. The various fungi were used to inoculate large tubes and jars of sterilized green-manured soil. The inoculated soil was planted to bacteria-free cotton-seed and soybeans. In the soil cultures no injury to germination was noted, except with culture 1. Here from 75 to 100 per cent of the seedlings were killed. Repeated tests with this unknown culture gave similar results. No injury to corn and wheat was noted from inoculations of culture 1, while soybeans and cotton were quickly destroyed.

Since the diseased root tips showed the presence of a Rhizoctonia-like fungus, it was arranged to study the effect of certain species of Rhizoctonia isolated from other sources. Two strains were employed—one isolated from potatoes, the other from alfalfa. The potato culture was secured from the Department of Plant Pathology of the Wisconsin Experiment Station; the alfalfa culture was supplied by Mr. Fred Jones, of the University of Wisconsin. Table XV gives the results of this test.

TABLE XV.—*Effect of Rhizoctonia spp. on the germination of cottonseed*

No.	Treatment and inoculum.	Germination.			Relative.
		1 week.	2 weeks.	3 weeks.	
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	None, sterilized. Uninoculated	75	80	80	100
2	1 per cent clover sterilized. Uninoculated	80	85	85	105
3	None, sterilized. Inoculated with *Rhizoctonia* sp. from alfalfa	60	70	70	86
4	1 per cent clover sterilized. Inoculated with *Rhizoctonia* sp. from alfalfa	.....	.....	.....	.....
5	None, sterilized. Inoculated with *Rhizoctonia* sp. from potato	80	80	80	100
6	1 per cent clover sterilized. Inoculated with *Rhizoctonia* sp. from potato	85	85	85	105

Rhizoctonia sp. isolated from alfalfa proved fatal to cotton seedlings. Two weeks after inoculation all of the young plants were dead. On the contrary, a species of Rhizoctonia from potato produced no noticeable injury to cotton seedlings. This difference in the action of the two strains of Rhizoctonia is very evident from Plate LXXXIII, figure 6, and the data in Table XV. A species of Rhizoctonia from alfalfa produced nearly the same effect on soybeans as on cotton, while the germination of corn was not affected.

A study of the optimum conditions for the growth of culture 1 and *Rhizoctonia* sp. from alfalfa showed that about 25° to 30° C. is the most favorable temperature for both of these fungi. The results of a previous study indicate that about 25° C. is the optimum temperature for the growth of the harmful factor. From the data as a whole, it seems very conclusive that the fungus of culture 1 and probably other fungi are the causative agents in the destruction of germinating seeds.

DESCRIPTION OF THE INJURY

Examination of the diseased seeds shows that the injurious factor probably does not attack seeds until after germination. Apparently the fungus attacks the primary root soon after germination. This occurs when the primary root is from ½ to 1 cm. long. The hyphæ pierce the walls of the host, entirely envelop the root, and often penetrate deep within the tissue. In the affected region the tissue loses its form, turns brown in color, and soon rots. Under the microscope these diseased seedling roots are surrounded by a dense mantle of hyphæ, which are often brown-colored.

RELATION OF GREEN MANURE TO INJURY OF OIL SEEDS

Although the evidence at hand does not warrant a definite conclusion, the author suggests the following as a possible explanation for the injury: The green tissue furnishes an excellent medium for the development of fungi. This is especially true in the first stages of decomposition. After one or two weeks in the soil the green manure undergoes certain changes which render it unsuited to the growth of the injurious fungi.

Just why oily seeds should be so sensitive to fungi is not known. It is possible that the oil partly changes to fatty acids in the process of germination (9, 10). According to Schmidt (19, p. 300–303), oil and fatty acids favor the growth of certain fungi. The fungus may produce a fat-splitting enzym—for example, lipase. This offers a possible explanation for the selective action of the injurious fungi for oil seeds.

SUMMARY

(1) Green manures may seriously injure the germination of certain seeds.

(2) This injury is brought about by the action of certain parasitic fungi.

(3) In the first stages of decomposition of green clover, numerous fungi develop. Some of these fungi are very destructive to seedlings.

(4) Oil seeds as a class are very easily damaged by fungi. Starchy seeds, on the contrary, are very resistant.

(5) Cotton seed and soybeans are examples of seeds extremely sensitive to green manuring. The germination of flax, peanuts, hemp, mustard, and clover is reduced in the presence of decomposing plant tissue, but not to as great a degree as that of cottonseed or soybeans. The germination of buckwheat, corn, oats, and wheat is not affected by green manures.

(6) The damage to oil seeds from green manures is confined largely to the first stages of decomposition. Experimental evidence shows that two weeks after green manure is added it does not cause any serious injury to the germination of oil seeds.

(7) Small applications of calcium carbonate seemed to increase the injury to germination.

(8) The rate of germination determines to a certain extent the degree of injury. Slow germination is marked by a high percentage of diseased seedlings.

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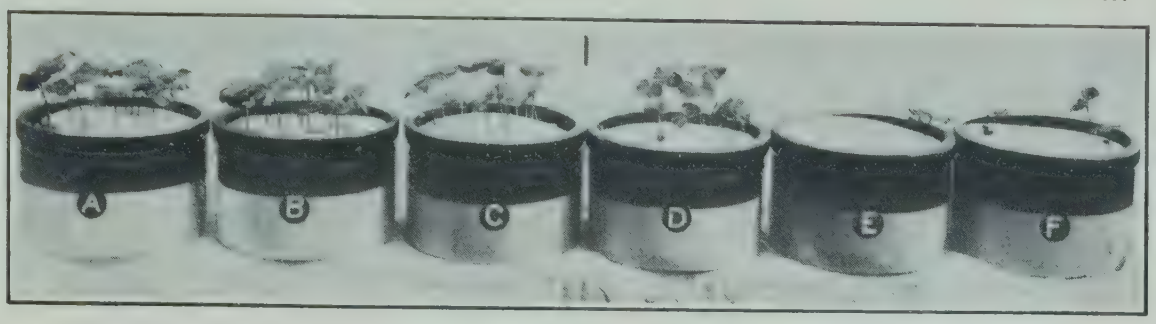
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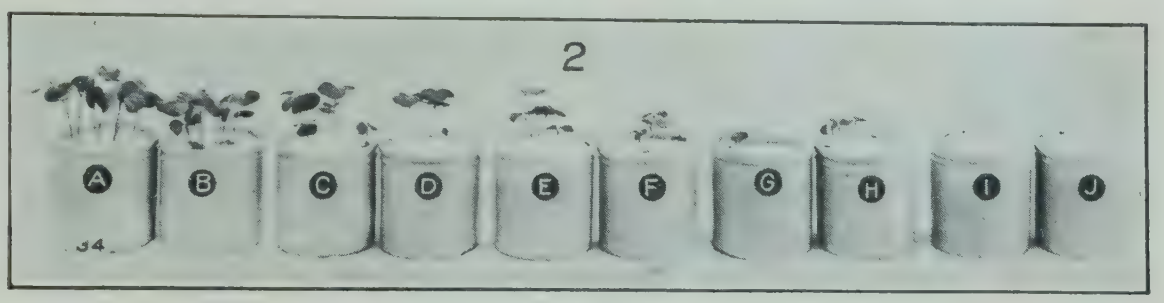
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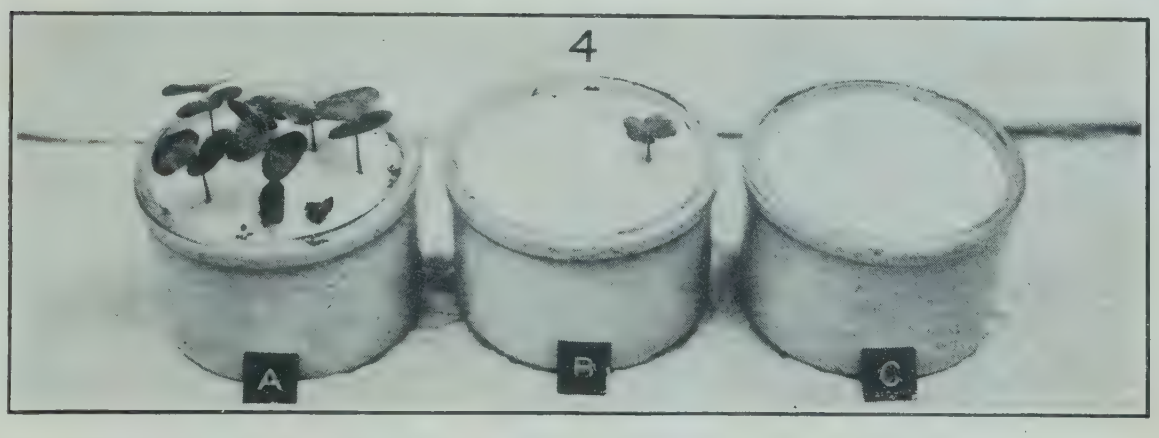
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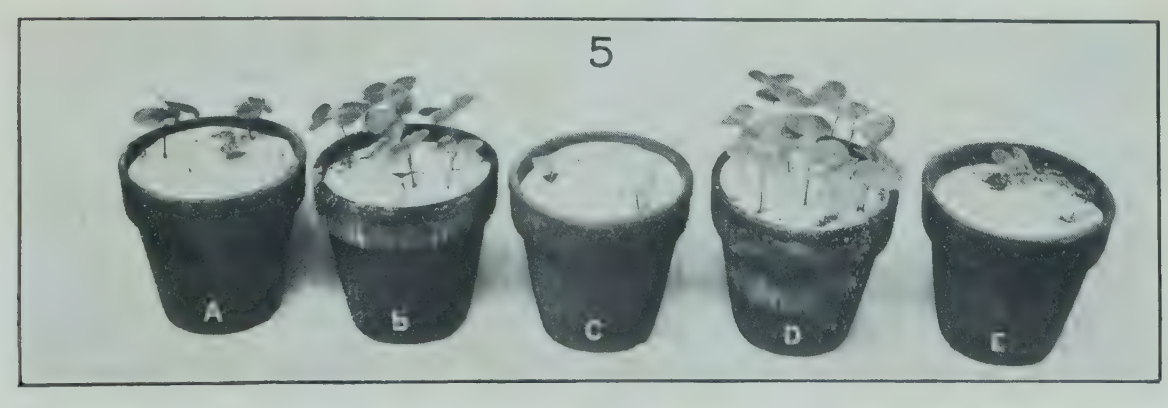
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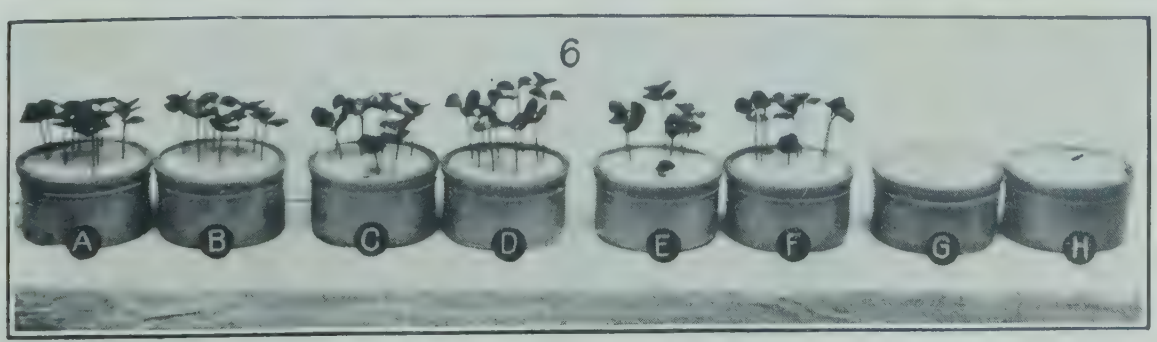
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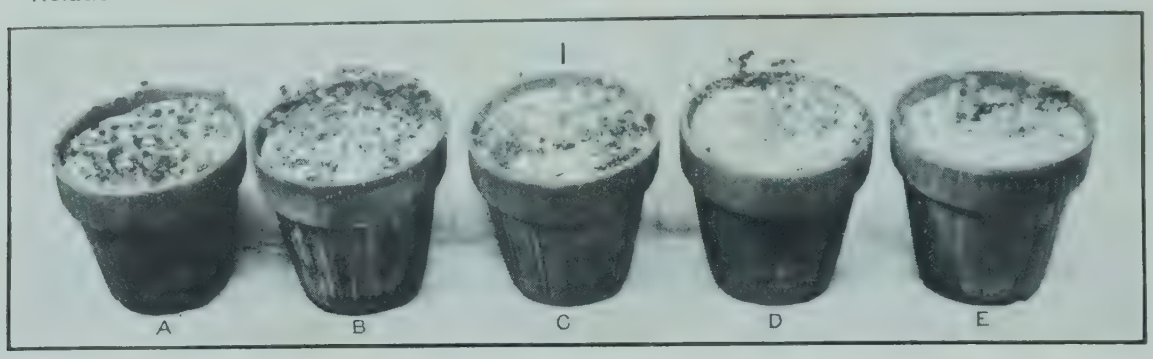
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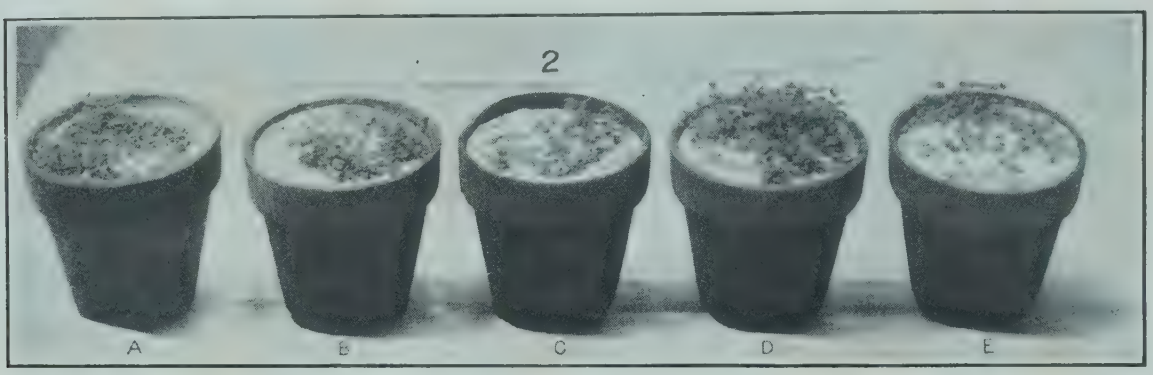
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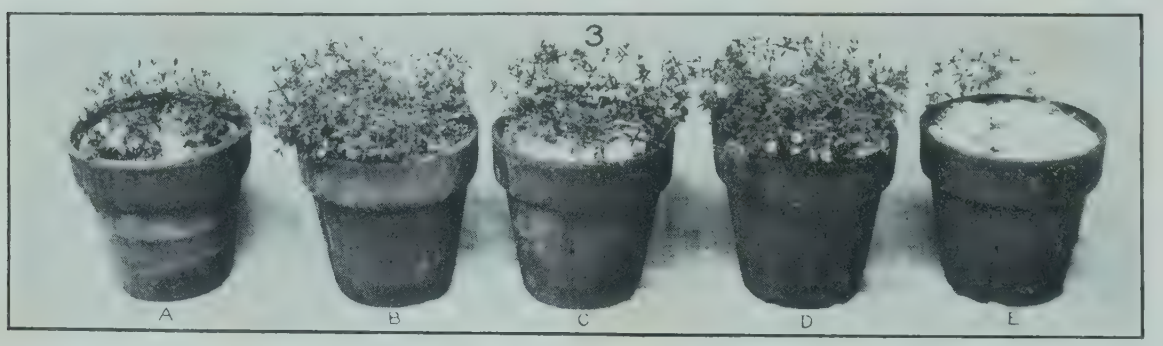
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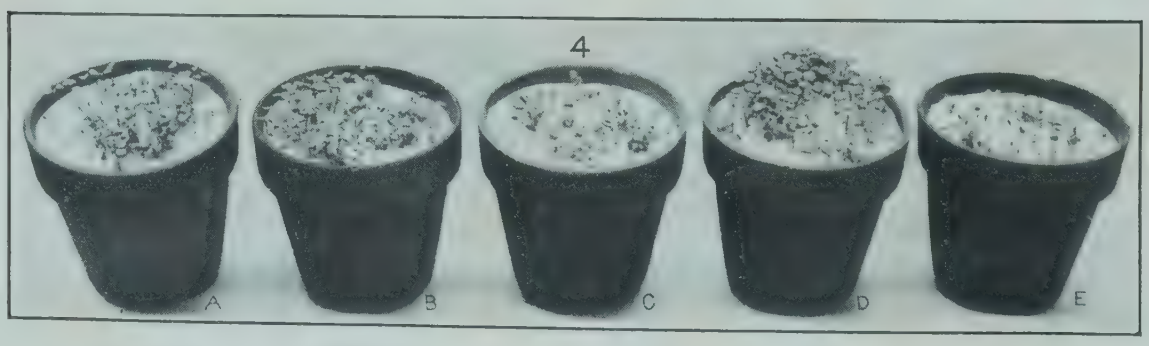
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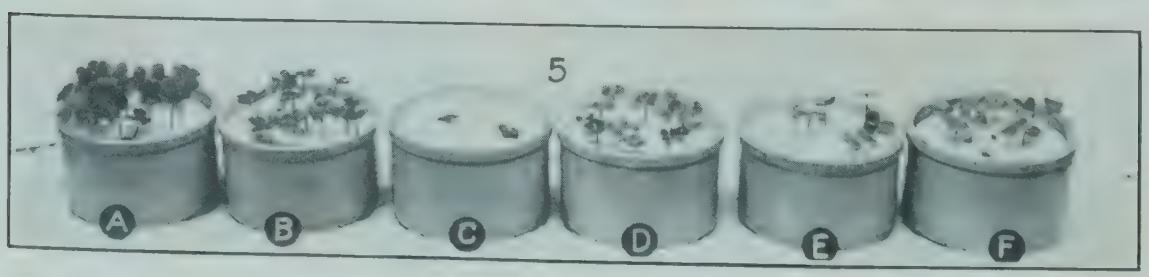
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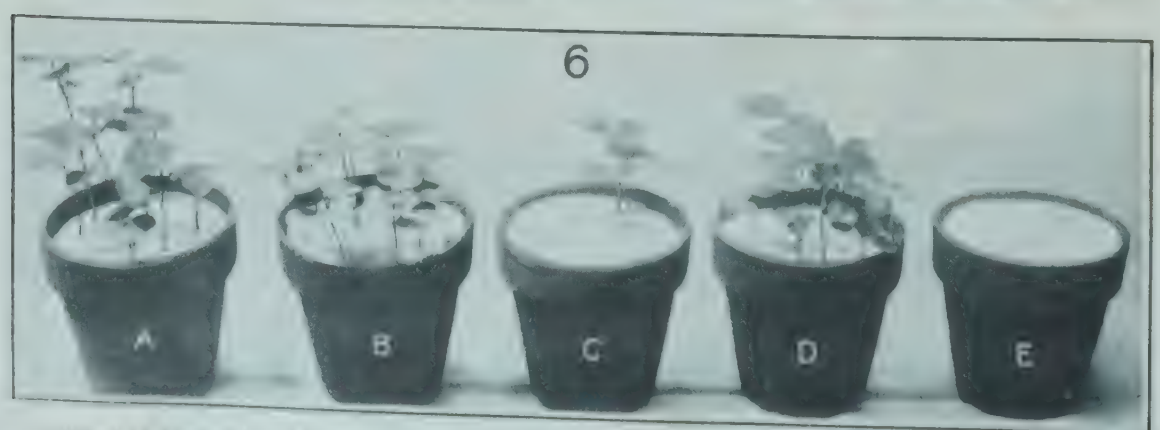
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A NEW SPRAY NOZZLE

By C. W. Woodworth,
Entomologist, Agricultural Experiment Station of the University of California

INTRODUCTION

A new principle has been discovered in nozzle construction whereby a flat spray can be produced with a uniform distribution of the water comparable to that of the hollow cone of spray from a cyclone nozzle. Hitherto all flat sprays have been of lenticular section, breaking up into fine mist on the sides and into relatively coarse drops in the center. It was observed that the flat spray produced by two impinging streams was at right angles to the original plane of motion of the two streams, but when the streams failed to meet squarely the plane was shifted and could, in fact, be moved through an arc of 180° with a very great change in the distribution of the water currents. It requires only a slight angular deviation to decrease very perceptibly the coarseness of the central drops, producing greater uniformity, and a position can be reached in which the coarsest drops are on the edge, those in the center therefore being the finest.

The principle finally discovered was that when two streams meet across half their section the resulting sheet of spray will be of practically uniform thickness throughout, occupying a plane 45° from the plane of the streams and finally breaking up into drops of great fineness and uniformity.

PRODUCTION OF SPRAY

There are two causes that may act in the production of spray particles: (1) Friction, which may cause an eddy along the edge of the stream sufficient to break the surface tension and allow the small eddying masses to fly off from the column of water; and (2) divergence of the direction of motion of the particles, resulting in the thinning out of the water mass in the form of irregular sheets until the surface film finally gives way and the sheet of water is suddenly converted into drops.

Both methods may be seen in the breaking up of the stream from a simple nozzle where, from the sides of the solid column of water, very minute particles of mist are given off, while the velocity and friction are great. With decreasing velocity farther on the eddies become larger, the mist gradually becomes coarser, and, finally, as the spread of the stream makes it break up into irregular sheets of water, the size of the drops produced by the second process results in an intermingling of drops of all sizes. At first the drops are very accurately graduated,

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those of the same size being produced at the same distance from the nozzle, but when the second process replaces friction as a cause of spray production, irregularity results, owing to the irregular shapes of the water sheets.

In a cyclone nozzle the stream at once diverges widely in the form of a hollow cone. Friction plays no part in the production of the spray, but the cone increases so rapidly in diameter that the liquid soon becomes a very thin sheet of unvarying thinness all the way around, and breaks into a uniformly fine mist. The uniformity may be assumed from the fact that on all sides the sheet extends an equal distance from the orifice before breaking into a spray, and experimentally can be shown to exhibit to an equally high degree both fineness and uniformity.

Figure 1 expresses in a diagrammatic form the facts shown by the photographs. The circles show the actual positions of the orifices in

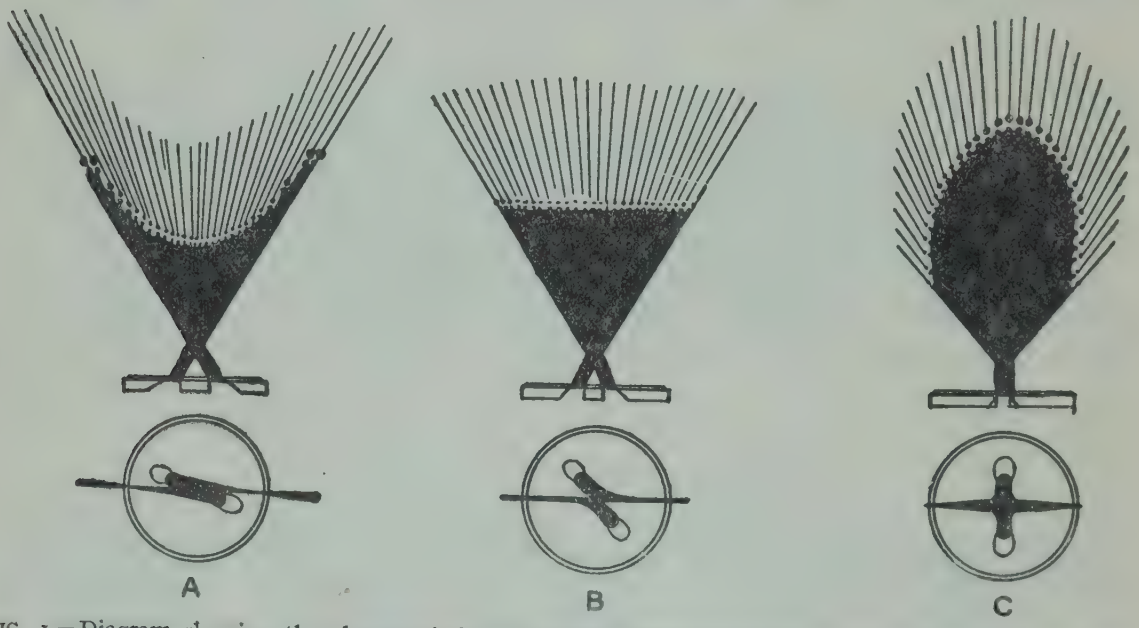


FIG. 1.—Diagram showing the characteristic differences between the three forms of impinging-stream nozzles.

each case and the black transverse marks give the effect of the impinging streams; the water remains thickest in the middle in *C*, thickest at the edges in *A*, while in *B* it is spread out evenly.

Above, the black portion indicates the water sheet, the sizes of the spots along the margin indicate the sizes of drops produced at these points, and the approximate velocity of the drops is shown by the length of the lines radiating from these spots.

SPRAYS PRODUCED BY IMPINGING STREAMS

The actual movement of the water in forming a spray through the impact of two streams is shown in Plate LXXXV and Plate LXXXVI, figure 1. It was not found practicable to secure the successive pictures with sufficient rapidity to show more than two steps in the forming spray, but by interpolating, a fairly satisfactory series was obtained. The

right-hand nozzle is of the common type where the streams impinge squarely. The middle nozzle is of the new type, but not strictly comparable with the former, since the streams come together at a broader angle, making a wider spray. Indeed, when the spray is under full pressure (Pl. LXXXVI, fig. 1) the spread is too wide, producing a lateral dribble and marginal fringe of spray. The left-hand spray is intermediate in angle and spread and gives the fish-tail effect.

The contrast is shown from the first illustration, the fish tail having thick marginal zones and the other two thick central zones, much shorter in the middle nozzle. In Plate LXXXVI, figure 1, where the spray sheets assume their normal proportions under high pressure, the large size of the white patch in the middle corresponds to the better final distribution of the spray particles. The irregularity of the spot shown on the left of this white patch is due to an irregularity in the orifice on the opposite side.

In Plate LXXXVI, figure 2, which shows the result of a sudden decrease of pressure, the character of the water sheets becomes especially evident, since they are increased greatly in size and the production of spray almost ceases.

ADVANTAGE OF A FLAT SPRAY

The cyclone nozzle leaves nothing to be desired in the way of fineness and uniformity of spray, but it has the disadvantage of making a ring of spray which surrounds instead of touching the object towards which the nozzle is directed. It is very difficult for one handling the nozzle to keep in mind the fact that the spray is strictly limited to the visible parts of the cone. A flat spray, on the other hand, reaches the point aimed at and is more available for treating branches of trees, for example, where the desire is to concentrate the spray on a line. For general spraying also the use of a flat spray, like the use of a flat brush for painting, gives uniform results more quickly and easily. For these reasons, while no other nozzle on the market produces a flat spray comparable in quality to the spray produced by the various types of cyclone nozzles, they are, nevertheless, more extensively used than the cyclone nozzles.

ADVANTAGE OF UNIFORMITY AND FINENESS

The use of nozzles of the flat type is generally acknowledged to be for the purpose of securing the flat shape of spray fan and is not a rejection of the principle that a uniformly fine spray is the most desirable. In fact, the use of these nozzles is generally associated with the use of high pressures, whereby the defects of a poor grade of nozzle are less apparent. The particular advantage of fineness is that it makes possible the even distribution of the spray material.

Fineness involves evenness. In a nozzle giving coarse drops, part of the material is in a finely divided state, and the improvement in a spray

nozzle comes through decreasing the size of all but the smallest particles and thus increasing the proportion of minute particles until, as in the cyclone nozzle, practically all of the material is in the most finely divided state and is therefore also uniform. This improvement can be produced by increasing the pressure or decreasing the size of the stream. Under the same pressure a nozzle with a large orifice gives coarser drops than a similar nozzle with a small orifice. Therefore, where a larger volume of spray is desired, it has been the practice to duplicate the nozzles rather than enlarge them, giving clusters of nozzles; but where high pressure is available, large nozzles, particularly those of the better type, may be used. With extreme pressures, such as were employed in the gipsy-moth work and in the walnut spraying in California, a nozzle of the poorest quality and rather large size has proved to be practical. In nearly all cases the desirability of fine and uniform sprays, in order to secure evenness of distribution, has been recognized. It is possible, however, that under some circumstances a driving spray may be desirable, and this can be secured only by the use of less efficient nozzles.

VARIATION IN FINENESS

The sizes of the smallest drops in a spray are not necessarily the same, particularly when made by the breaking up of a sheet of water. By a change in the proportions of the eddy chamber in a cyclone nozzle or by a change in spraying pressure the diameter of the cone at the point of breaking can be changed, and the drops will remain uniform, but will be of a different size than before. In the new type of nozzle here described the angle of impact and the spraying pressure exert similar effects, and a series of nozzles can be produced covering much the same range obtainable in a cyclone nozzle and distinguishable by the width and length of the fan.

Only relatively small drops in the spray in either case are obtained, and these show great uniformity, the variation in size being inside of rather narrow limits.

The new type of nozzle is the form in which the spray is in a plane inclined at the angle of 45° from the plane of the impinging streams, but between that and the usual style, having the spray in a plane 90° from that of the streams, there is the possibility of any number of intermediate forms that present any desired degree of uniformity in the size of the drops. Should a compromise nozzle giving a driving spray with greater uniformity than in the existing nozzles be desired, it can readily be constructed. The same could be secured by a disproportion between the sizes of the two streams, and in this case the coarser portion would be at one edge instead of at the center of the fan. This form might be desirable for some spot-spraying for scale insects, and it might be desirable to have a means of controlling the size of one of the streams.

WHERE THE NEW NOZZLES ARE IMPRACTICAL

Because the spray must first be separated into two streams in this type of nozzle it becomes particularly liable to clogging and should not be used for any spraying where there is any such tendency—e. g., with Bordeaux mixture.

Most of the spray materials now used, however, are clear solutions and give no trouble in the nozzle.

LONG- AND SHORT-DISTANCE NOZZLES

When the angle is widest between the impinging streams, the angle of the fan is likewise widest, the drops finest, and the carrying distance of the spray the shortest.

An acute angle between the impinging streams produces a very narrow spray which carries a longer distance, but may perhaps finally reach nearly as great a width as that of the rapidly spreading short-distance spray.

Some prefer a long-distance nozzle and use it close to an object, as where spot spraying on a tree trunk is desired. The new type of nozzle lends itself very readily to adjustment to any degree of distance, from the shortest to nearly the longest found in spray nozzles.

ADJUSTMENT

Any form of two-stream nozzle, like that known as the calla, or lily, nozzles, can be quickly converted into a nozzle of the new type by the use of a reamer, slightly enlarging the two apertures on opposite sides by working the instrument obliquely to the surface of the nozzle and trying it from time to time until the spray sheet stands at 45°.

The same process will enable one to adjust a nozzle at any time should it wear irregularly enough to change the angle of the spray fan. The shape of the fan is a good index of the correct adjustment. If the angle is just right, the fan is triangular; if less than 45°, it is shortest in the center and the spray is coarser at the ends. If the angle is more than 45°, the fan is longest in the center and the spray coarsest at this point.

With care the reamer can be so used as to effect the change in the stream without enlarging the hole at the surface, and, therefore, not changing the volume of discharge. It may be possible to change the angle of the spread of the fan by reaming out beneath on the side adjacent to or opposite the other hole. One should continually try a nozzle while adjusting it, so as not to carry the work too far.

SUMMARY

(1) A new principle employed in nozzle construction will produce a flat spray with the qualities of a cyclone nozzle.

(2) A uniform sheet of water breaking along its edge produces drops of uniform size.

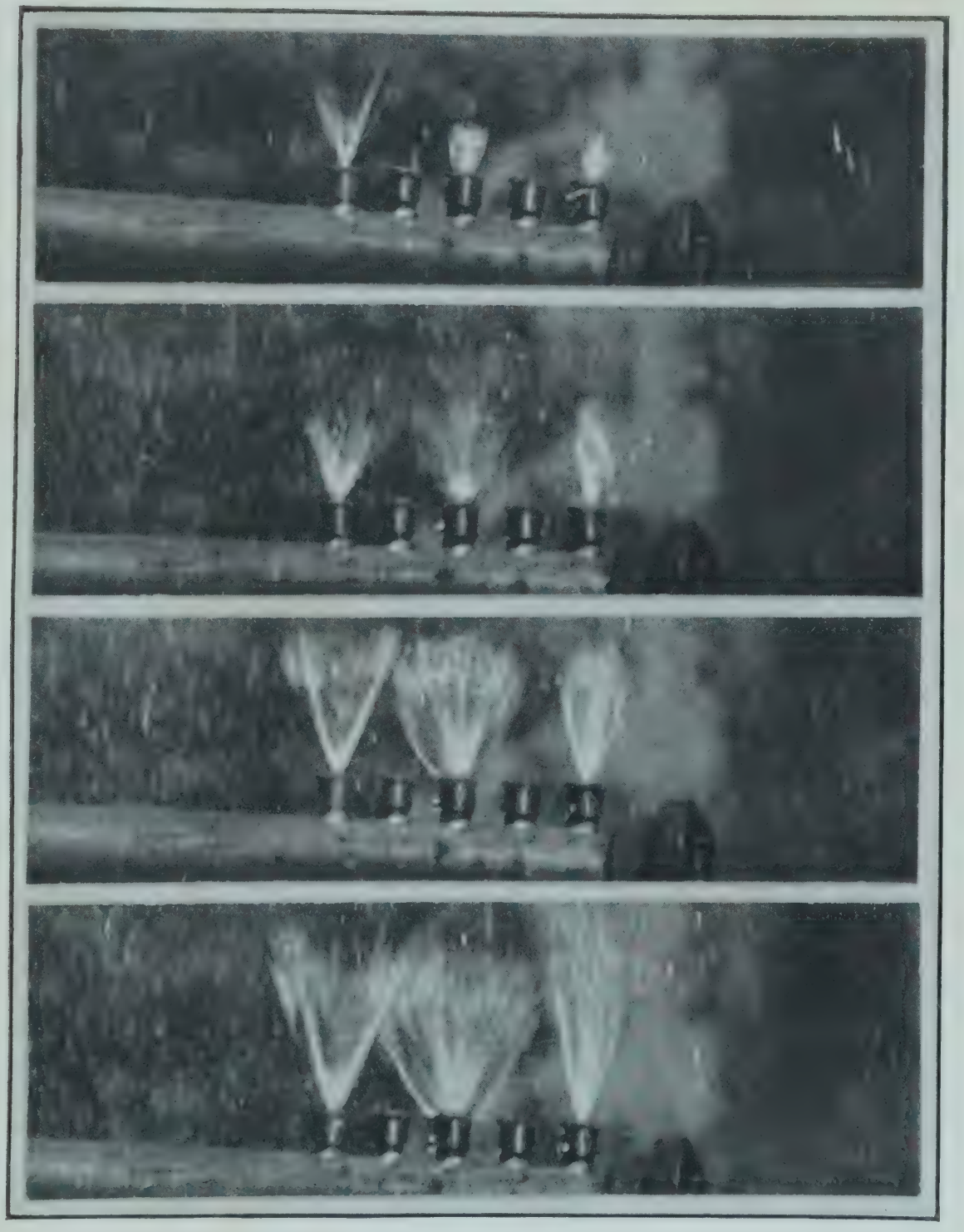
(3) A flat spray is more easily directed and produces a more uniform distribution than the cone of spray from a cyclone nozzle.

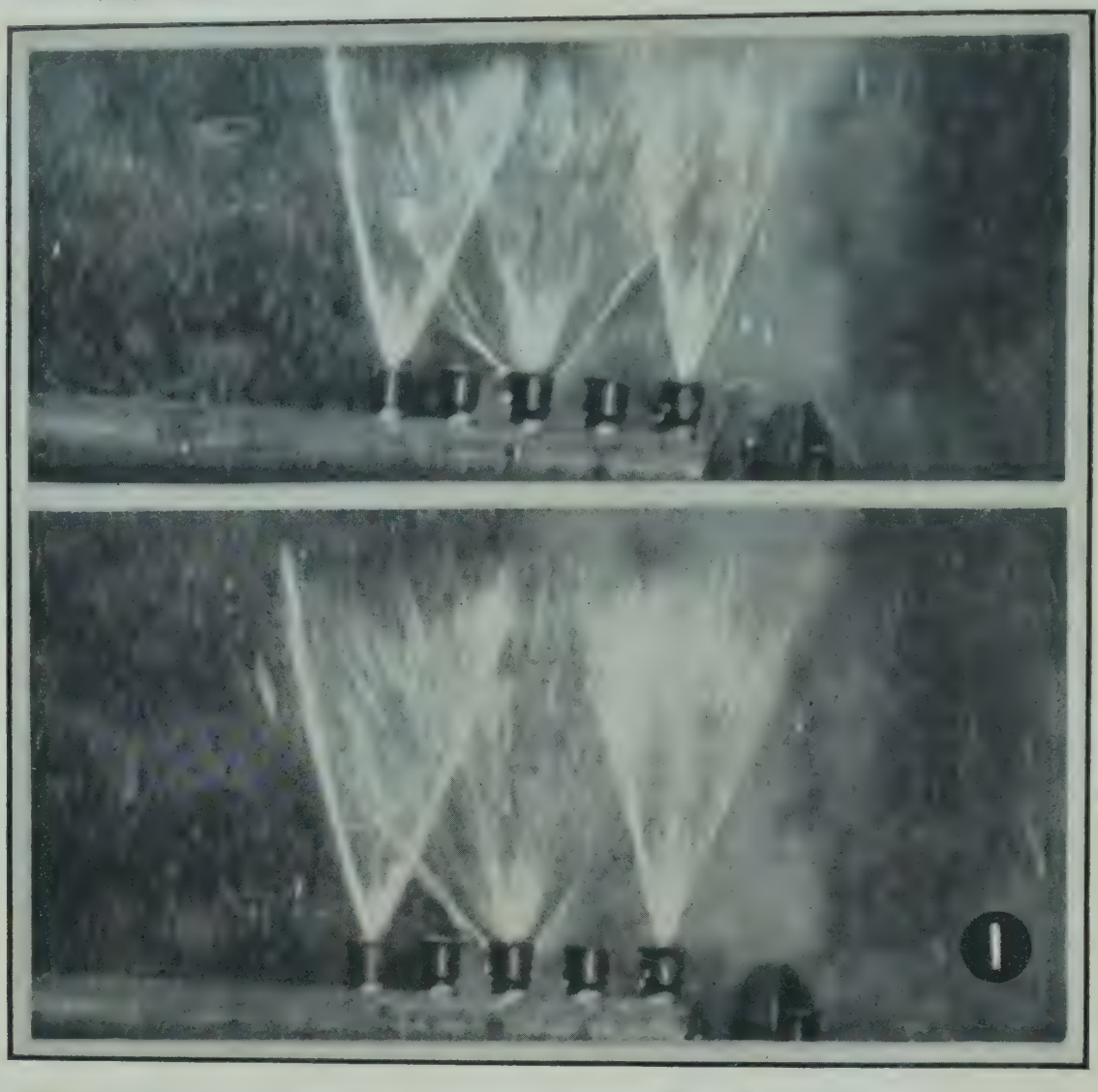
(4) Uniformly fine drops of spray aid in securing uniformity of distribution.

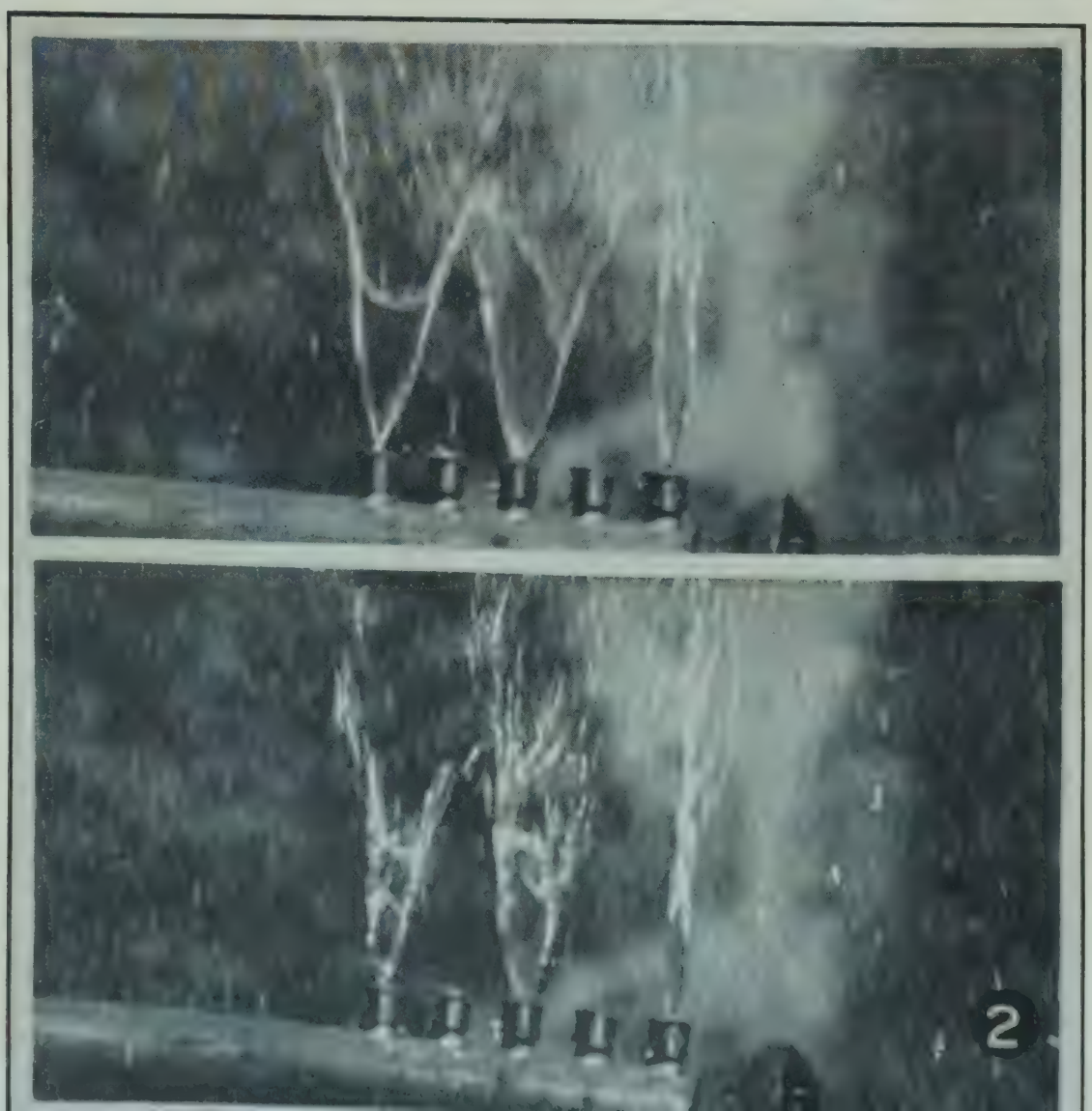
(5) The new nozzle allows some variation in size of spray.

(6) It also may be made into a long- or short-distance nozzle.

(7) It can be easily constructed by modifying existing nozzles and may be adjusted if it becomes worn.



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A NEW INTERPRETATION OF THE RELATIONSHIPS OF TEMPERATURE AND HUMIDITY TO INSECT DEVELOPMENT

By W. Dwight Pierce,

Entomological Assistant, Southern Field Crop Insect Investigations, Bureau of Entomology

INTRODUCTION

Upon the proper interpretation of the laws of climatic control of life rests the solution of many practical problems, and inasmuch as all plant and animal life reacts to climate in the same general manner it is apparent that the study of the climatic control of insect development may throw light upon the problems of all other forms of life. It has been apparent to some workers in the field of ecology that our so-called laws of effective temperature were deficient in many respects. A large number of phenomena were not properly explained by any known theory. It is with the hope that the present interpretation may come closer to the truth that this paper has been prepared.

Biologists for years were laboring with the theory of a fixed zero of effective temperature for all life, and only recently was it accepted that each species might have a different zero. It has been the custom to determine the thermal constant for any given activity by multiplying the number of effective degrees accumulated above the effective zero in daily units of mean temperature by the time in which the given phenomenon took place. The noneffective low temperatures were eliminated, but not the time in which they were experienced. Inasmuch as most workers were located in north-temperate climates, where high noneffective temperatures seldom occur, it had not occurred to them that some high temperatures might not be effective and that there was another boundary to the effective zone besides the zero. These high temperatures and the time in which they are experienced must be eliminated. In addition to all of these errors in method, there has been no correlation of the humidity factor until very recently, although now many workers are trying to solve the part played by this factor.

The principal data upon which the writer has based his studies include records of thousands of individual boll weevils (*Anthonomus grandis* Boh. and *A. g. thurberiae* Pierce), made by the members of the boll-weevil force under the direction of Mr. W. D. Hunter and the writer at various localities in Texas, Louisiana, and Arizona throughout the period of years from 1902 to 1915. At each place where biological notes were made a thermograph-hygrograph record was kept, and this record was

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checked twice daily by maximum and minimum thermometer and sling-psychrometer readings. The means of temperature and humidity are based upon these records. In addition to the natural records, a series of artificial-cold experiments were conducted at various times, and the writer recently conducted an extensive series of artificial-heat experiments with definite humidity control in order to determine the effects of heat.

EXPERIMENTAL METHODS

Before venturing to present this new interpretation the writer has thoroughly discussed it with many prominent workers, and it is now proposed for more extensive criticism and elaboration.

To express the relationship of the two factors, temperature and humidity, to insect metabolism, development, and activity, a temperature scale may be marked off on the vertical line of a sheet of plotting paper and a humidity scale from left to right on the horizontal line. There are, for any given insect, definite boundaries of atmospheric temperature and humidity within which the life of the species revolves. There is a temperature below which, even for the shortest time, life is impossible—the absolute minimum fatal temperature. There is also a temperature above which, even for a moment, life is impossible—the absolute maximum fatal temperature. Absolute dryness is more or less prohibitive of life and so is absolute humidity, or saturation, although some insects may be adapted better to withstand extremes of humidity than others. It is quite possible that the boundaries of humidity may be 0 and 100 per cent, or infinitesimally close thereto.

The diagrammatic figure sought, however, has four definite absolute boundaries—the maximum and minimum temperatures and humidities.

Within the limits which we have thus defined there exist conditions under which all the activities of the species reach their maximum efficiency. It has been conceived by most writers that this maximum efficiency was reached at a definite point known as the optimum. It seems more likely that it will prove to be a zone of humidities and temperatures of more or less restricted area. A careful study of the records of any species, charting for the time required for each activity and the temperature and then similarly for humidity, will disclose temperature and humidity points of maximum efficiency. With the boll weevil these points lie approximately near 83° F. and 65 per cent of relative humidity.

ZONES OF CLIMATIC RELATIONS

At any ordinary humidity, starting with the absolute minimum fatal temperature, as the temperature increases a longer and longer time of exposure is required to kill, until a point is reached at which life continues indefinitely. This zone of temperatures has been called the zone of fatal temperatures.

As the temperature continues to rise it passes through a zone of ineffective temperatures, known commonly as the zone of hibernation, which the writer will shortly prove to be an inappropriate term. At the lowest temperatures in this zone complete dormancy without metabolism is found; but as the temperature increases a gradual approach to sensibility is noted, first metabolism, next movement, and then the necessity of feeding. The point at which metabolism or growth begins at a given humidity is the zero of effective temperature.

As the temperature increases above this zero the activity is at first very sluggish, but becomes more and more active until the so-called optimum is reached, and from this point upward the temperatures cause less and less activity, inducing stupor and finally sleep or coma.

At the point of coma begins the zone of ineffective temperatures formerly known as estivation. With the increase of temperature sleep becomes more and more sound until a point is reached at which death occurs after long exposure. At this point begins the zone of high fatal temperatures at which death occurs at shorter and shorter periods until it is instantaneous at the absolute maximum fatal temperature. This completes the vertical cross section of the figure desired. A statement regarding these vertical zones was first published by the Bureau of Entomology in 1912.[1]

In the past, however, the fact that a similar horizontal cross section at any temperature can be made, starting at absolute dryness and reading toward absolute humidity, has not been recognized. In this manner are shown zones of fatal dryness, dryness causing stupor, increasingly effective humidity, the most effective humidity, decreasingly effective humidity, excessive humidity causing drowsiness, and finally fatal humidity, at least under certain conditions of exposure.

In the case of the boll weevil the resulting figure is a series of concentric ellipses centered about the optimum and with diagonal axes. On the accompanying diagram the main details of the relations of temperature and humidity to the boll weevil are brought out. Only a few of the more salient records are included. The development in buds (cotton squares) is based upon hundreds of individual records, but is not reported in detail. The outer lines are much less definitely located than the inner ones, but whatever their actual location the picture would be substantially the same.

EFFECTIVE TEMPERATURE

Workers who have used the zero of effective temperature in their studies will note that, according to the present theory, the zero when charted is an elliptical curve representing a different point at each degree

[1] Hunter, W. D., and Pierce, W. D. Mexican cotton-boll weevil. 62d Cong., 2d Sess., Sen. Doc. 305 (U. S. Dept. Agr. Bur. Ent. Bul. 114), p. 125–128. 1912.

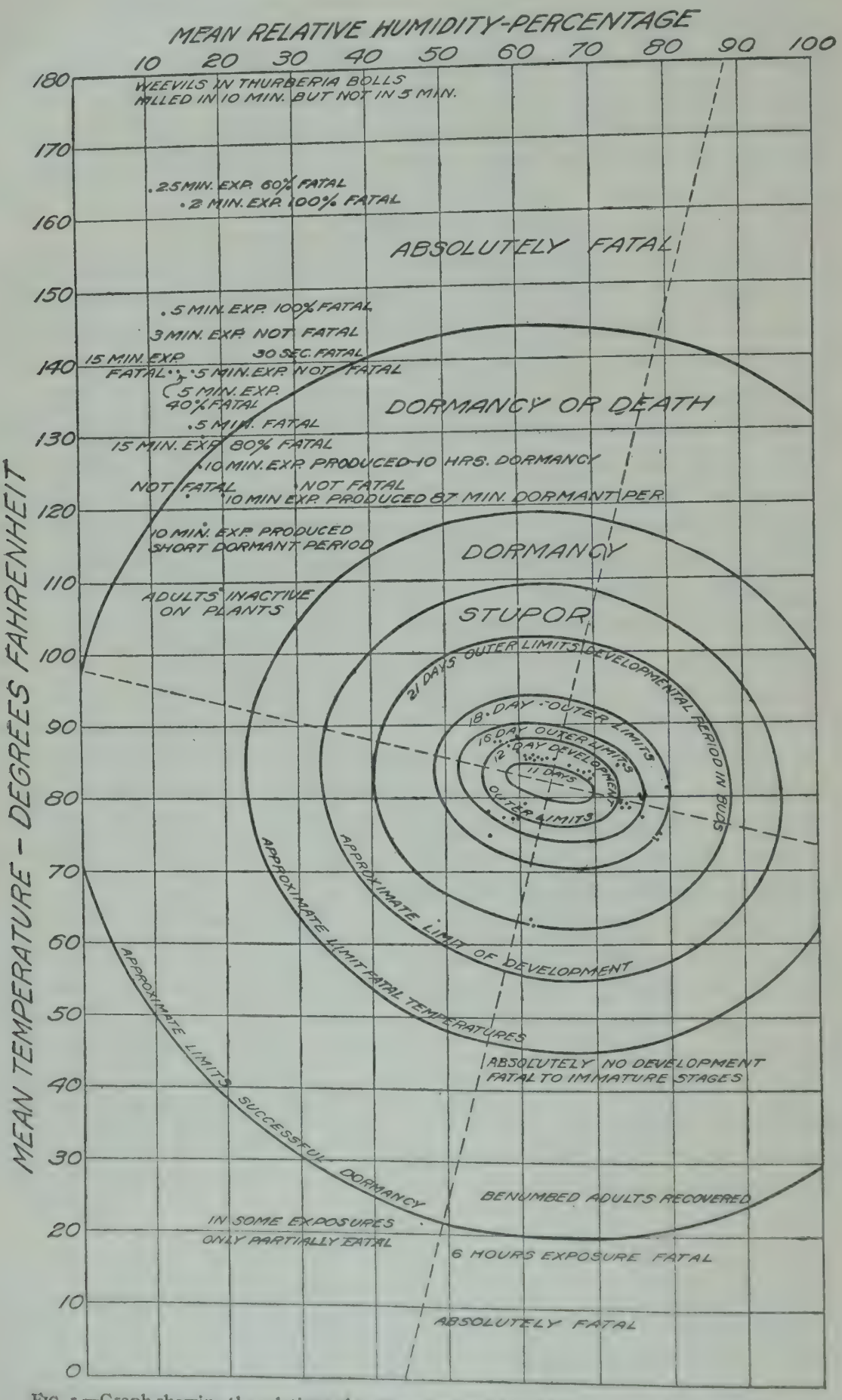
MEAN RELATIVE HUMIDITY-PERCENTAGE
10 20 30 40 50 60 70 80 90 100
MEAN TEMPERATURE - DEGREES FAHRENHEIT
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
WEEVILS IN THURBERIA BOLLS KILLED IN 10 MIN. BUT NOT IN 5 MIN.
.25 MIN. EXP. 60% FATAL
.2 MIN. EXP. 100% FATAL
ABSOLUTELY FATAL
.5 MIN. EXP. 100% FATAL
3 MIN. EXP. NOT FATAL
15 MIN. EXP. FATAL
30 SEC. FATAL
5 MIN. EXP. NOT FATAL
5 MIN. EXP. 40% FATAL
.5 MIN. FATAL
DORMANCY OR DEATH
15 MIN. EXP. 80% FATAL
10 MIN. EXP. PRODUCED 10 HRS. DORMANCY
NOT FATAL
NOT FATAL
10 MIN EXP. PRODUCED 87 MIN. DORMANT PER.
10 MIN. EXP. PRODUCED SHORT DORMANT PERIOD
DORMANCY
ADULTS INACTIVE ON PLANTS
STUPOR
21 DAYS OUTER LIMITS DEVELOPMENTAL PERIOD IN BUDS
18 DAY OUTER LIMITS
16 DAY OUTER LIMITS
12 DAY DEVELOPMENT
11 DAYS
OUTER LIMITS
APPROXIMATE LIMIT OF DEVELOPMENT
APPROXIMATE LIMIT FATAL TEMPERATURES
ABSOLUTELY NO DEVELOPMENT FATAL TO IMMATURE STAGES
APPROXIMATE LIMITS SUCCESSFUL DORMANCY
BENUMBED ADULTS RECOVERED
IN SOME EXPOSURES ONLY PARTIALLY FATAL
6 HOURS EXPOSURE FATAL
ABSOLUTELY FATAL

FIG. 1.—Graph showing the relations of temperature and humidity to cotton boll-weevil activity

of humidity. Because of the difficulty of computing this zero, the writer has been requested to describe his method of computing effective temperatures.

The first step is to tabulate all records of a given mean percentage of humidity on a single sheet. The zone of effective temperatures must be worked out separately at each degree of humidity. Only by a laborious series of testings can the first zero be approximated, unless the worker finds it by a fortunate chance. The total effective temperature is the criterion by which we finally know when we have rightly defined the limits of the zero. This is known as the thermal constant and is the multiple of the mean of the effective temperatures (between the zero and the absolute), figured in day units, by the time in which these effective temperatures were experienced. Noneffective temperatures, whether high or low, and the time in which they were experienced must be eliminated. The zone of effective temperatures will be finally reached for any given humidity when the

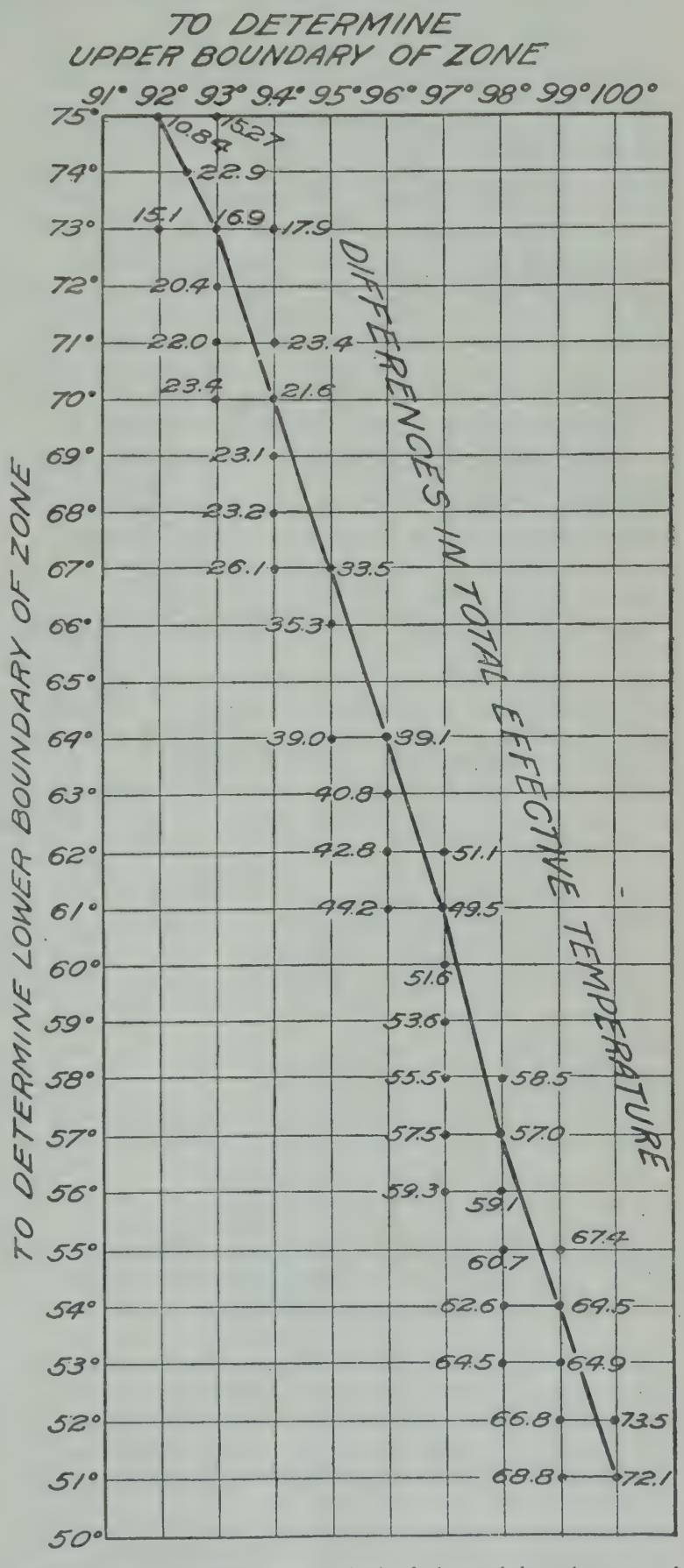

FIG. 2.—Graph showing the method of determining the zone of effective temperatures at a humidity of 56 per cent.

difference in the total effective temperatures is reduced to a minimum. At the start some arbitrary zero must be chosen and the effective temperatures computed above this. Then it is necessary to remove degree by degree at the top or bottom and note each time whether the difference in the total effective temperatures becomes larger or smaller. This process may be charted so that the general tendency can be seen. The figures found in the writer's attempt to establish the zone of effective temperatures for the boll weevil at 56 per cent humidity will illustrate the manner in which the points desired were ascertained. These results are presented in figure 2, and it will be seen that the first tentative zone chosen was 51° to 100° F. By much testing it was narrowed to within the limits of 75° to 92° F., for which the optimum is practically 83.5°.

Having obtained the limits of the zone, the records of development in cotton squares at a mean humidity of 55.9 per cent to 56.9 per cent, made at Victoria, Tex., in 1913, by Mr. B. R. Coad, of the Bureau of Entomology, are as shown in Tables I and II.

TABLE I.—*Records of development of Anthonomus grandis at Victoria, Tex., in 1913, at a humidity of 55.9 to 56.9 per cent*

Experiment.	Mean humidity.	Date of oviposition.	Time of maturing.	Actual period of development.	Number of weevils observed.		Total weevil days.	Actual temperature.		
					Male.	Female.		Absolute maximum.	Absolute minimum.	Mean.
	Per cent.			*Days.*				° *F.*	° *F.*	° *F.*
1	56.1	July 27	Aug. 9	13	6	2	104	104	73.2	88.2
2	56.4	July 26	Aug. 8	13	1	3	52	104	73.2	88.2
3	56.6	July 27	Aug. 10	14	.....	1	14	104	73.2	88.2
4	56.9	July 27	Aug. 11	15	.....	1	15	104	73.2	88.2
5	55.9	May 22	June 7	16	1	.....	16	95.5	54.5	78.2
Mean....	56.2	.....	.....	.....	Total. 8	7	201	.....	.....	.....

TABLE II.—*Records of development of Anthonomus grandis at Victoria, Tex., in 1913, in the zone of effective temperatures, 75° to 92° F.*

1[a] Experiment.	2[a] Number of weevils.	3[a] Mean humidity.	4[a] Humid time units.	5[a] Period experiencing effective temperature.	6[a] Total effective weevil days.	7[a] Mean effective temperature.	8[a] Effective thermal units.	9[a] Mean daily effective temperature. units.	10[a] Total effective temperature.	11[a] Humidity plus effective temperature.
		Per ct.		*Days.*		° *F.*			° *F.*	° *F.*
1	8	56.1	448.8	8.19	65.52	83.8	670.4	8.8	72.0	139.9
2	4	56.4	225.6	8.18	32.72	83.8	335.2	8.8	71.98	140.2
3	1	56.6	56.6	8.86	8.86	83.6	83.6	8.6	76.1	140.2
4	1	56.9	56.9	9.52	9.52	83.7	83.7	8.7	82.82	140.6
5	1	55.9	55.9	9.95	9.95	82.6	82.6	7.6	75.6	138.5
Total....	15	.....	843.8	.....	126.57	.....	1,255.5	.....	.....	.....
Average.	.....	56.2	.....	8.43	.....	83.7	.....	8.7	73.3	139.9
Difference...	.....	.....	.....	.....	.....	.....	.....	1.2	10.84	2.1

[a] Column 4 is product of columns 2 and 3. Column 5 is computed from the actual records. Column 6 is the product of 2 and 5. Column 8 is the product of 2 and 7. Column 9 is 7 minus the zero (75° F.). Column 10 is the product of columns 5 and 9. Column 11 is the sum of columns 3 and 7.

From these tables it will be seen that the effective period of development is from 8 to 10 days, averaging 8.43 days, while the actual development ranged from 13 to 16 days. It is noticeable that in all of the records the maximum as well as the minimum temperatures ran outside of the zone of effective temperatures. The total effective temperature ranged from 72° to 83° F., with 73.3° as the weighted mean and with a total difference of only 10.84°, a very small difference.

It is not necessary in this paper to give the further details of the zone of effective temperatures at other humidities. The determination of the zone for the next percentage of humidity is much less difficult, because it must be just a little narrower or a little wider than already determined. As the axis is diagonal, the upper and lower bounds will depart at a different rate. After several points have been determined, the axis can be located and then the figuring becomes very simple. It must be noted that every hour of effective temperature has its cumulative effect, even in the winter time.

ZONE OF INACTIVITY

One of the results of the acceptance of the present interpretation will be the necessity of discarding the conception of separate zones of hibernation and estivation. Physiologists have demonstrated that the effects of heat and cold on metabolism are alike. The writer has frequently noticed in field work the impossibility of differentiating between a frozen and a heat-killed boll-weevil larva. Prof. G. G. Becker, of Arkansas Agricultural College, several years ago observed that the fall army worm, *Laphygma frugiperda* S. and A., had two periods of activity and two of inactivity every day in the hot days in the Ozarks. Activity began in the morning and continued until the early part of the afternoon, when the heat caused the worms to be inactive for several hours. They then again became active during the early hours of the night, but the nights were cold and the worms became inactive until morning. The phenomena of a year were reproduced day by day. Inactivity due to cold in the summer time can not properly be called hibernation.

In Arizona the boll weevil is now native on wild cotton (*Thurberia thespesioides*). It normally breeds in the bolls in the fall, becoming adult by December 1, but remains in its cell throughout the cold winter and the warming spring. In some canyons there is a spring rainy season and *T. thespesioides* has a spring fruiting season. In these localities the moisture also releases the weevils from their cells and they begin breeding. A dry season follows and the weevils go to sleep. In other canyons the spring is not wet and the plants and weevils are inactive until the regular rainy season in August, when the long rest is broken. In some canyons the weevils therefore have two resting periods during the year, and in other canyons they are at rest from fall until summer. It not infrequently happens that the August rainy season does not materialize, and under

such circumstances the weevils stay in their cells and the plants remain dormant until the next year or perhaps for several years. As evidence of this the writer kept several of these weevils over 500 days without food or water, and one lived 626 days, dying only when moisture invaded the room where it was kept.

Hunter, Pratt, and Mitchell [1] record the unusual ability of larvæ of *Hermetia chrysopila* Loew, a cactus scavenger fly, to withstand long periods of drought. Larvæ in various stages of development were kept for more than 15 months without food and developed readily later when food was supplied. The very leathery integument seems to protect the insect against desiccation, and in other ways the larva has evidently adapted itself to long periods of waiting for favorable food, which, in the arid regions, depends upon the infrequent rains. Both of these instances are more properly resting periods due to dryness than to cold or heat.

NOMENCLATURE OF CLIMATIC EFFECTS ON LIFE

As charted, there are three elliptical zones which express the three principal effects of climate on life, viz, activity, inactivity, and death. The zone of activity may be known as the "thermopractic" zone (*Θερμός*, meaning heat, plus *πρακτικός*, meaning effective). The zone of inactivity may be known as the zone of "anesthesia" (*ἀναισθησία*, meaning insensibility). The zone of death may be known as the "olethric" zone (*ὀλέθριος*, meaning deadly). The region of greatest activity may be known as the "practicotatum" zone (*πρακτικώτατον*, meaning most effective).

Many phases of climatic effects have been differentiated, and medical literature abounds in words descriptive of these effects. For some effects no words are available. The writer has thought it best to present a complete and consistent system of nomenclature, based on the Greek, using all words already in the language, and only supplying new words where none are now available.[2]

It may be convenient to refer to the most effective temperature or the most effective humidity, in which cases we may use the words "thermopracticotatum" or "hygropracticotatum."

The awakening from sleep is termed "anastasis" (*ἀνάστᾶσις*). We can therefore speak of "thermanastasis" and "hygranastasis," depending on whether the awakening is caused by a change of temperature or a change of humidity.

Heat, moisture, dryness, or cold added to the "practicotatum" will cause sluggishness. We have to indicate this condition the term "nochelia"

[1] Hunter, W. D., Pratt, F. C., and Mitchell, J. D. The principal cactus insects of the United States, U. S. Dept. Agr. Bur. Ent. Bul. 113, p. 38–39. 1912.

[2] New Standard Dictionary. 1913.

Gould, G. M. An Illustrated Dictionary of Medicine, Biology and Allied Sciences . . . ed. 6, with . . . Sup . . . 1633, 571 p., Philadelphia, 1910.

(νωχέλεια, meaning sluggishness) and can show the type of sluggishness by the addition of a prefix, as "thermonochelia," "hygronochelia," "xeronochelia," and "rhigonochelia."

At least three of these factors produce under extreme conditions a stifling sensation, and we may express this by the terms "thermopnigia," "xeropnigia," and "hygropnigia" (πνίγος, meaning stifling).

The stifling sensation ends in complete insensibility, or anesthesia, and this word may be modified to express the cause, as in the term "thermanesthesia," "hygranesthesia," "xeranesthesia," and "rhiganesthesia."

Death from heat is known as thermoplegia (πληγή, meaning stroke), while from excessive moisture it may be known as "hygroplegia," and from freezing, as "rhigoplegia." Death from drying is known as "apoxeraenosis" (ἀποξηραίνω, meaning to dry up).

The determination of locomotion by heat is called "thermotaxis," and movement brought about by heat is called "thermotropism."

Unusual sensibility to heat is called "thermalgesia" and "hyperthermalgesia." The ability to recognize changes of temperature is "thermesthesia," and its extreme is designated as "thermohyperesthesia," abnormal sensitiveness to heat "stimuli." Fondness for heat or requiring great heat for growth is called "thermophilic," while resistance to heat is called "thermophylic." Rapid breathing, owing to high temperature, is designated as "thermopolypnea," contraction under the action of heat as "thermosystaltic," adapting the bodily temperature to that of the environment as "pecilothermal," and a morbid dread of heat as "thermophobia."

The life after apparent death, called "anabiosis," is exemplified in such cases as that of the Hermetia larvæ mentioned above.

Pain from the application of cold is called "cryalgesia," abnormal sensitiveness to cold "cryesthesia," and a morbid sensitiveness to cold "hypercryalgesia."

PRACTICAL APPLICATIONS

Many practical measures will result from the further study of climatic relations to life. A few of these may be indicated.

One of the most effective measures for the control of the cattle tick is pasture rotation based upon the possible duration of life of the seed tick without an animal host. As this period varies with the season, it is necessary to know the climatic laws under which this species reacts.

The fall army worm advances across the country and again retreats in complete accord with changing temperatures. The proper fixation of the zone of effective temperature may make it possible to plan the planting of winter crops to avoid damage.

The cotton boll weevil must have food up to the time that it enters hibernation. Early harvesting and destruction of stalks before the low temperatures set in offer one of the most satisfactory methods of control.

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